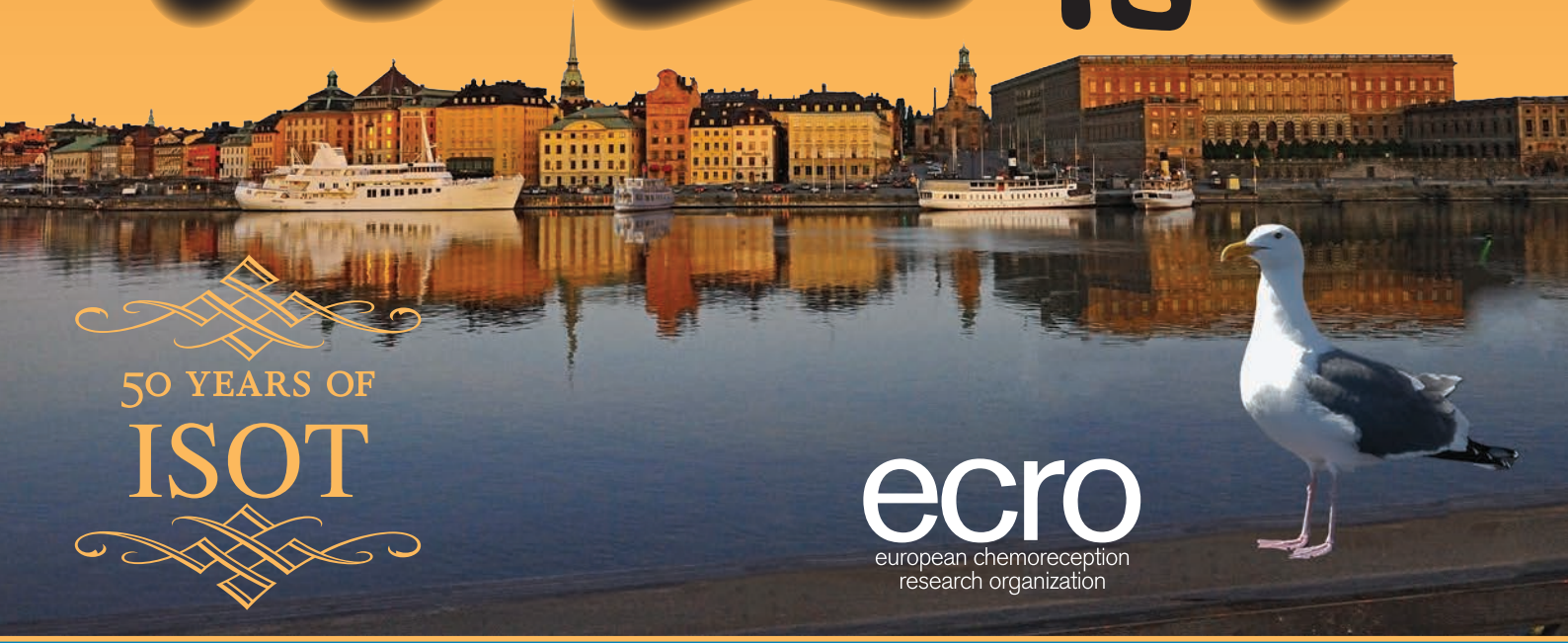
XVI International Symposium on Olfaction and Taste

Stockholm, Sweden, June 23–27 2012

ISOT 16



Abstracts

Welcome

Welcome to Stockholm and ISOT XVI

We celebrate 50 years of cutting edge research in taste and olfaction by returning to the birthplace of ISOT. ECRO welcomes you to some days of intense exchange of information via talks, posters and discussions. ISOT offers a unique opportunity for all of us active in different areas of chemosensory science to meet up and tell about what has happened in the labs and in the field the last four years. ISOT is a meeting place not only for the host organization ECRO, but also its sister organizations: the Japanese Association for the Study of Taste and Smell (JASTS) and the Association for Chemoreception Sciences (AChemS). We also welcome our friends from the Australasian Association for Chemosensory Science (AACSS). Thanks to the support from ECRO and its sister organisations over the world, to a number of sponsors and to the work by the session organizers we have gathered an outstanding collection of chemosensory scientists.

The program of ISOT XVI spans over all areas of chemosensation.
We have made an effort to bring in the flavors of ecology and ethology, but also a feeling for the stimulus (i.e. some chemistry) into the program. We hope that you will enjoy the complete bouquet!

We trust that you will find Stockholm to be a welcoming and friendly city, and hope that you will find time to enjoy the sights and experience the atmosphere. We are entering the town just after the midsummer celebrations so you might find the Saturday a bit slow, but then it should pick up.

ISOT XVI could not have been organized without the support of our sponsors. The Linnaeus program "Insect Chemical Ecology, Ethology and Evolution (IC-E3)", the Swedish University of Agricultural Sciences, the Max Planck Society, the Swedish Research Council (VR) and the Swedish Research Council Formas all contributed significantly.

Special thanks goes to our corporate Gold Sponsors and symposium sponsors Biotec Holding, Fondation Jean-Marie Delwart and the Ajinomoto Corporation. Fondation Jean-Marie Delwart also funds the Delwart Prize in Higher Olfactory Processing. Thanks also to our Silver Sponsors Bayer Crop Science, Bedoukian Research, ChemCom, Coca Cola and Givaudan and to our additional sponsor Syntech GmbH. Thank you also to Merja Immonen and Akademikonferens for their help in organizing the meeting. Finally, we would like to express our special gratitude to Mr Jean-Marie Delwart personally for his continued support for the chemosensory sciences and for his involvement in ISOT XVI particularly.

Bill S Hansson
Meeting Organizer

For the organizing committee
Mikael A Carlsson • Susanne Erland • Bill S Hansson • Ylva Hillbur • Marcus Stensmyr

Special thanks

We would like to send special thanks and appreciation to the following companies and organizations for their generous support of the 2012 ISOT/ECRO Meeting.

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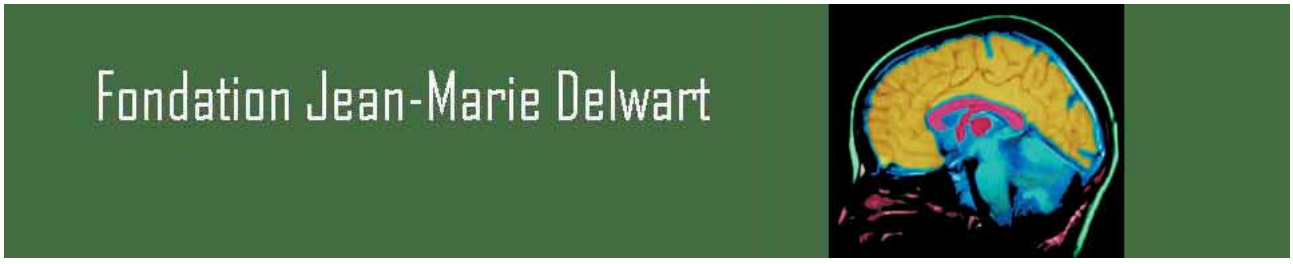

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We express special thanks to the Polak Foundation for supporting ECRO fellowships for young researchers. The Polak awards are funded by the Elsje Werner-Polak Memorial Fund in memory of our niece gassed by the Nazis in 1944 at age 7: Ghislaine Polak and the late Ernest Polak.

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SPP ISOT student travel grants

We would like to express our sincere gratitude to DFG Schwerpunktprogramm (SPP) 1392: Integrative Analysis of Olfaction

BRILL Martin (Rössler lab)
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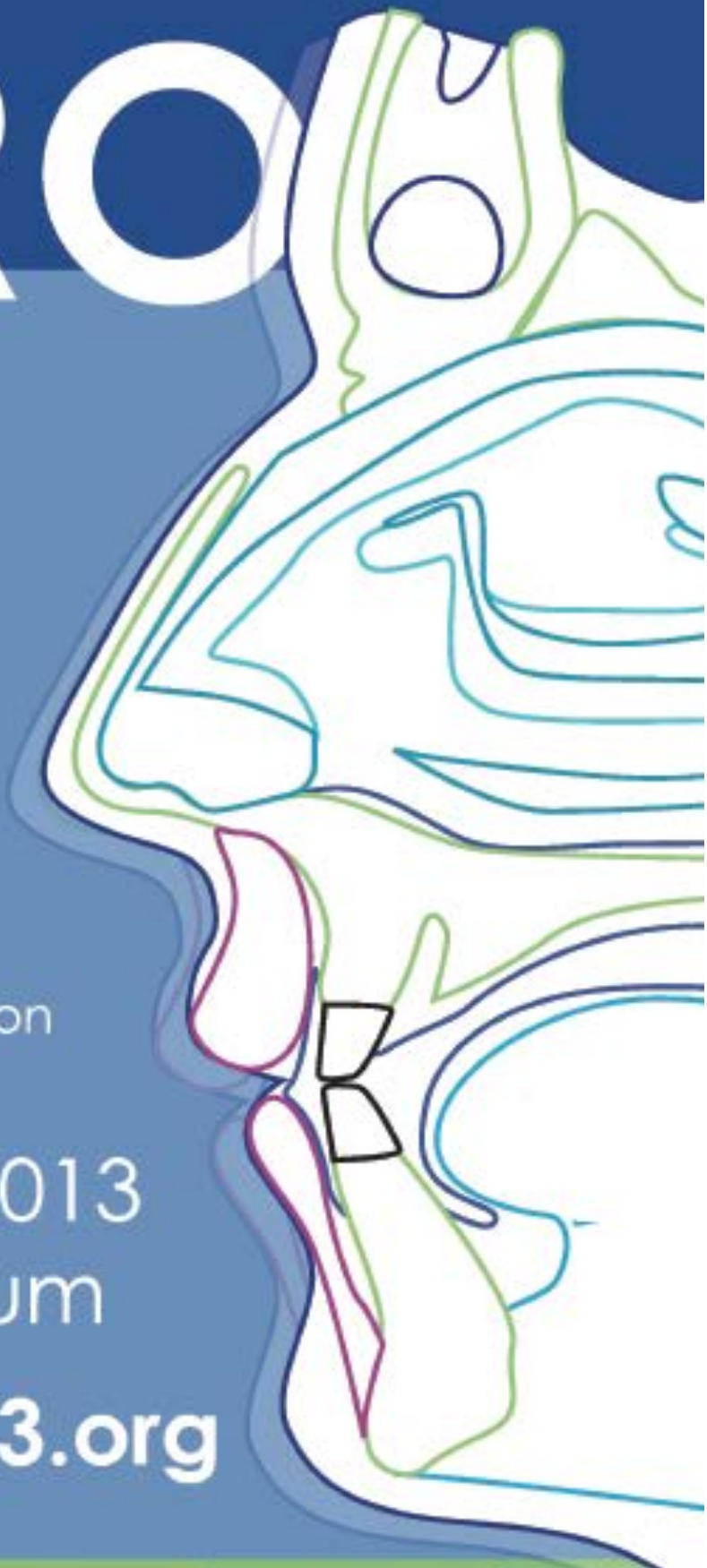

European Chemoreception
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26-29 August 2013
Leuven - Belgium

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Program at a glance

	23 June	24 June	25 June	26 June	27 June
08.30-09.30		Plenary lecture: *Genes, connectomes, and decisions: using fixed circuits to drive flexible behaviors*	Plenary lecture: *Mating switches olfactory coding and preference – Linnaeus Lecture*	Plenary lecture: *Olfactory mechanisms in mammals – Delwart Lecture +Delwart Prize*	Plenary lecture: *Taste recognition in Drosophila*
09.30-10.00		Coffee	Coffee	Coffee	Coffee
10.00-12.00	Registration starts	*Olfactory and taste circuits*	*Plasticity and modulation in olfactory systems - Linnaeus Symposium*	*Higher olfactory processing - Delwart Symposium*	*Molecular and neural basis of taste detection*
		Interspecific chemointeractions			*Odor memory and perception: cells to circuits*
		Robotics and artificial chemosensors			*Evolution of chemosensory systems*
12.00-13.00		Lunch	Lunch	Lunch	Closing
13.00-15.00	-Welcome session -ISOT 1962 -Keynote lecture: *The biology of mammalian taste: from the tongue to the brain*	*Human olfaction*	*Modulation of the olfactory system - Linnaeus Symposium*	*Higher olfactory processing – Delwart Contributed Symposium*	Excursions start at 13.00 in front of the Stockholm Waterfront.
		Central mechanisms of taste learning and memory	*Gustation*	*Taste and beyond - integration of nutrient sensor functions in oral cavity and gut - Ajinomoto Symposium I*	
		Chemosensory initiated mating behaviour	*Mixed session*	*Toward a genetic basis for human olfaction*	
15.00-15.30	Coffee	Coffee	Coffee	Coffee	
15.30-17.30	*The other noses – the vomeronasal organ, the septal organ and the Grüneberg ganglion*	*From odorant receptor to glomerulus*	*Olfactory receptors, ligand interactions and transduction mechanisms*	*Olfactory neuroethology*	
	Coding of taste across mammals: from the tongue to the cortex	*The stimulus – odor space and chemometrics*	*Human olfaction*	*Preference for umami taste controlled by chemical senses - Ajinomoto Symposium II*	
	Chemosensory receptors in non-chemosensory tissues	*No taste, no smell: when the chemical senses are lost*	*Insect-host interactions*	*Aquatic olfaction*	
17.30-	Welcome reception	Poster session I	Poster session II	Banquet 19.30 Buses depart 18.45	

Locations: A1 C2 C3 M1 Balcony Vasa Museum Entrance

Symposium 21 "Molecular and neural basis of taste detection" Wednesday 27 June

Taste system in fish

Keiko Abe[1], Shinji Okada[1] and Takumi Misaka[1]

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Fish as a vertebrate has a taste signaling system which is similar to those of humans and rodent animals. Fish as well have amino acid receptors T1Rs and bitter taste receptors T2Rs in the link with their down-stream molecules, G proteins, PLCβ2 and TRPM5.

We have observed that medaka fish's taste nerves respond to acids and also that the pH thresholds of the responses are reflected in the behaviors. This suggests that fish and mammals have common PKD family molecules as a candidate sour taste receptor. We have actually found the expression of mfPKD2L1, a PKD2L1 ortholog in a subset of taste bud cells and neurons surrounding central canal of the spinal cord in medaka fish. Although there are no PKD1L3 ortholog in fish genome, our observations using double-labeling in situ hybridization revealed that PKD1L2 orthologs are co-expressed with PKD2L1 in medaka fish. PKD2L1/PKD1L2b co-express in taste buds and PKD2L1/PKD1L2a co-express in neurons surrounding central canal. It is conjectured that the responding pH range of fish PKD2L1 varies depending on its partner that constitutes a heterodimer together. We also found that taste bud PKD-positive taste cells in fish as well as those in mammals differ from T1Rs/T2Rs-containing PLCβ2-expressing taste cells.

To visualize the neuronal circuit connected to these sensory cells, we generated a transgenic medaka expressing the trans-synaptic tracer lectin, wheat germ agglutinin (WGA), under the control of medaka PLCβ2 gene regulatory region. Immunohistochemical analysis of adult transgenic fish sections revealed that multiple WGA-positive neurons were detected in the cranial sensory ganglia, known gustatory relay nuclei, and other brain regions. These results provide us with a basis for understanding a common logic of the gustatory information-processing system in vertebrates.

Poster session I Poster #1

Behavioral and sensory modulation of olfactory responses in starved *Drosophila*

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Environmental factors as e.g. temperature and diurnal rhythm are well known to modulate olfactory responses both on physiological and behavioral levels. We hypothesized that internal factors can also modulate olfactory responses. We tested, whether a change of the feeding status causes olfactory modulation and used the olfactory circuit of *Drosophila* as a well-established model. We show that starved flies have a lowered behavioral response threshold to odors, and that the sensitivity of OSNs is in parallel increased. The change in behavioral and physiological responsiveness is not restricted to food odors, but is found also for non-food odors like cis-vaccenyl acetate (CVA; pheromone) and benzaldehyde (repellent). Behavioral and physiological levels of responsiveness can be reversed by feeding previously starved flies. To investigate the molecular players behind the increased sensitivity of starved flies we analyzed gene expression patterns within antennae and brains of starved and fed flies. A microarray analysis revealed some genes to be up- or down-regulated after starvation either in the antenna, in the brain or at both levels. With these results as a base and by using the *UAS-Gal4* and the *UAS-shibire* system we could show that neuropeptides and biogenic amines are involved as modulatory factors in starvation-caused modulation.

The increased behavioral response of starved flies and their increased olfactory sensitivity demonstrate that the *Drosophila* olfactory circuit is modulated by the feeding state. We could identify several molecular factors that are involved in this modulation, and show that sensitivity is increased for both food and non-food odors. Hence, starved flies seem to be tuned not only to locate potential food sources from long distance, but in addition to evaluate food quality and the presence of conspecifics with higher sensitivity.

This project was funded by the Max Planck Society

Poster session I Poster #159

An anatomical spotlight on the mouse vomeronasal organ

Tobias Ackels[1], Daniela Fluegge[1], Susanne Lipartowski[1] and Marc Spehr[1]

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As the peripheral sensory structure of the accessory olfactory system, the vomeronasal organ (VNO) predominantly detects social chemosignals. Interaction with conspecifics, including social and sexual behavior, aggression and mate choice are thus fundamental functions of the vomeronasal system. Therefore, the basic goal of this study is to provide a multi-level structural perspective of the mouse VNO. Here, we take advantage of diverse tissue preparation methods in combination with multiple microscopy and digital reconstruction techniques. From in vitro preparations of single dissociated sensory neurons to acute in situ sections of the intact sensory epithelium, and from encapsulated VNO dissections to whole-mount en-face preparations, our approach sheds light on the cell morphology and structural anatomy of the VNO. Each preparation is suited for different electrophysiological and/or imaging techniques, thus, providing a versatile experimental 'toolkit' for comprehensive physiological analyses of VNO function.

Poster session II Poster #160

The functional evolution of mammalian odorant receptor orthologs

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It is generally assumed that orthologs—genes related via speciation—retain equivalent function in closely related species. However, this assumption remains largely untested. The mammalian odorant receptor (OR) repertoire is an attractive model to study the functional evolution of orthologs because OR genes have been subjected to extensive gains and losses between species, presumably caused by adaptation of the olfactory system to the environment. Yet, many ORs have clear orthologs in closely related species. We investigated the functional properties of primate and rodent OR orthologs expressed in heterologous cells to determine how well gene orthology predicts functional characteristics. Using human and mouse ORs with previously identified ligands, we cloned 18 OR orthologs from chimpanzee and rhesus macaque and 17 mouse-rat orthologous pairs that are broadly representative of the OR repertoire. We functionally characterized the responses of OR orthologs to a wide panel of odors and found similar ligand selectivity but differences in response magnitude. 87% of human-primate orthologs and 94% of mouse-rat orthologs showed differences in receptor potency (EC50) and/or efficacy (dynamic range) to an individual ligand. Additionally, we found that orthologs responded to a common ligand 82% of the time, while human receptors of the same subfamily responded to the common ligand 41% of the time. Our results suggest that while OR orthologs tend to show conserved ligand selectivity, their potency and/or efficacy dynamically change during evolution even in closely related species. These functional changes in orthologs provide a platform for examining how the evolution of odorant receptors can meet species-specific demands.

Poster session II Poster #168

Milk is time! Young mouse pups prefer milk odour from early rather than from late lactation

Syrina Al Aïn[1], Laurine Belin[1], Bruno Patris[1] and Benoist Schaal[1]

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Milk is rich in odour-active compounds and mammalian newborns eagerly respond to its effluvium, especially when it comes from conspecific females. In the mouse, milk is known to fluctuate compositionally during the first postpartum days and then between weeks 1 and 2 of lactation, suggesting that its odorous profile may change accordingly. The present study aims to assess whether there are periods in lactation during which milk would carry stronger olfactory

potency for neonatal and young mice. To assess whether such hypothetic fluctuations in milk are olfactorily detectable to offspring, mouse (Balb-c) pups of differing ages [postnatal (P) days 0, 2, 6 and 15] were exposed to the headspace of milks collected from females in differing lactational stages [lactation (L) days 0, 2, 6, and 15]. The pups were assayed in a series of paired-choice tests opposing either murine milk and a blank stimulus (water), or 2 milks obtained from females differing in lactational stage.

It appeared first that pups of any age were attracted by the odour of milk regardless of its lactational stage. Second, when milks from different lactational stages were paired two by two, P2 and P6 pups oriented for a similar amount of time to the odors of L2 and to L6 milks, but significantly less to the odor of L15 milk. Next, P15 pups were attracted as much by contemporaneous L15 milk than by the milks from earlier (L2, L6) lactational ages. Finally, ongoing assays with P0 pups indicated longer orientation to L0 milk than to milks from later stages.

Thus, P2 and P6 pups exhibit selective attraction to the odour of milks collected up to L6. Conversely, P15 pups show non-selective attraction to any milk odour. Such a developmental shift in the response to milk odour may be explained either by alterations in the odor properties of milk, by shifting pup chemosensory abilities, or by the combination of these processes. Finally, the developmentally-changing response to milk odour may further be explained in terms of postnatal variation in the adaptive necessity to express a selective response to milk.

Poster session II Poster #80

Combinatorial activation and repression by seven transcription factors specify *Drosophila* odorant receptor expression

Mattias Alenius[1], Shadi Jafari[1], Liza Alkhori[1], Alex Schleiffer[2], Anna Brochtrup[3] and Thomas Hummel[3]

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The mechanism that specifies olfactory sensory neurons to express only one odorant receptor from a large repertoire is critical for odor discrimination but poorly understood. Here, we will present the first comprehensive analysis of regulators of odorant receptor expression in *Drosophila*. A systematic, RNAi-mediated knock down of most of the predicted transcriptions factors identified an essential function of *acj6, E93, Fer1, onecut, sim, xbp1* and *zf30c* in the regulation of more than 30 odorant receptors. These regulatory factors are differentially expressed in antennal sensory neuron classes and specifically required for the adult expression of odorant receptors. A systematic analysis not only reveals that combinations of these seven factors are necessary for receptor gene expression but also a prominent role for transcriptional repression in preventing ectopic receptor expression. Such regulation is supported by bioinformatics and OR promoter analyses, which uncovered a common promoter structure with distal repressive and proximal activating regions. Thus, our data provide insight into how combinatorial activation and repression can allow a small number of transcription factors to specify a large repertoire of neuron classes in the olfactory system.

Poster session I Poster #187

Calcium-activated chloride channels in the apical region of mouse vomeronasal sensory neurons

Asma Amjad[1], Michele Dibattista[2, 1], Devendra K Maurya[1], Claudia Sagheddu[1], Giorgia Montani[3], Roberto Tirindelli[3], Anna Menini[1]

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The rodent vomeronasal organ plays a crucial role in several social behaviors. Detection of pheromones or other semiochemicals occurs in the microvilli of vomeronasal sensory neurons, where the binding of molecules to vomeronasal receptors leads to the influx of sodium and calcium ions mainly through the transient receptor potential channel 2 (TRPC2). However, the physiological roles of the increase in intracellular calcium concentration are not completely

understood. In this study, we produced a rapid increase in calcium concentration in the apical region of isolated mouse vomeronasal sensory neurons with flash photolysis of caged calcium and measured calcium-activated currents with the whole-cell voltage-clamp technique. On average, a large inward calcium-activated current of -261 pA was measured at -50 mV, rising with a time constant of 13 ms. Ion substitution experiments showed that this current is anion selective. Moreover, the chloride channel blockers niflumic acid and DIDS partially inhibited the calcium -activated current. Furthermore, we showed by immunohistochemistry that the calcium-activated chloridechannels TMEM16A/anoctamin1 and TMEM16B/anoctamin2 are expressed in the microvilli where they co-localize with the TRPC2 transduction channel. Therefore, we conclude that microvilli of mouse vomeronasal sensory neurons have a high density of calcium-activated chloridechannels that may play an important role in vomeronasal transduction.

Poster session II Poster #2

Larval olfactory experience influences host plant choice in adult oviposition and mate finding in a moth.

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Selection of suitable host plants is essential for all herbivorous insects. However, the mechanisms that predispose insects to choose some plants instead of others are still poorly understood, particularly in polyphagous insects. We have studied host plant preferences to five plants in male mate search and female oviposition of the polyphagous moth, *Spodoptera littoralis*. The experiments revealed robust preference hierarchies, mostly overlapping between the sexes. We also demonstrated strong effects of larval experience on host plant preference, where the larval host plant species was generally elevated to the most preferred in both sexes, without otherwise affecting the overall hierarchy. Our results show that host plant choice is guided by a stable innate hierarchy, which can be modified by larval experience. Such mechanisms may reduce costs associated with polyphagy and contribute to functional plasticity in plant choice and adaptation to novel environments.

Furthermore, larvae reared on artificial diet, but exposed to volatiles during the larval period from a host plant, showed adult preference for these plants. Thus, exposure to plant volatiles during the larval stage was sufficient to induce the adult preference and no contact with the host plant was needed. The role of larval experience in host plant selection has been debated and questioned, and the studies have been limited to female behaviour. Our results show the importance of larval experience for both male mate search and female oviposition and that the preference change depends on olfactory stimuli.

Poster session I Poster #81

Expansion of the odorant receptor family and reduction in the gustatory receptor family in the Hessian fly: - An adaptation to a stressful lifestyle?

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Chemicals in the environment mediate most insect behaviors crucial for fitness, such as selecting mates or hosts. The Hessian fly (Diptera, Cecidomyiidae) is a serious pest of wheat in most wheat growing areas in the world. The short adult life (1-2 days) means that flies are forced to accomplish host and mate finding within a very restricted timeframe, which puts a high demand on the chemical senses.

The insect odorant receptors (ORs) and gustatory receptors (GRs) belong to a large superfamily of chemoreceptors. The ORs detect volatile molecules, whereas the GRs perceive CO_2, sugars, bitter tastants, and cuticular hydrocarbons. We annotated 122 ORs and 27 GRs from the Hessian fly genome. Comparing the ORs with ORs of *D. melanogaster* and *A.*

gambiae, we find drastic expansions of two major OR lineages in the Hessian fly. The largest expanded lineage contains 71 Hessian fly ORs, and not a single OR from the other species. Many of these ORs share very high amino acid identity (up to 100 %) and are located close on chromosomes, indicating recent duplication events.

In contrast, we found a considerable reduction in GRs, by at least half, relative to the other available Diptera with genome sequences. Hessian fly contains all three CO_2 receptors and hence should be able to detect CO_2. There is, however, a reduced set of candidate sugar receptors, 3 versus 8 in the other fly lineages, and no ortholog of the GRs implicated in cuticular hydrocarbon perception in *Drosophila*. For both the ORs and GRs, the Hessian fly appears to have lost numerous lineages that might have been present at the base of the Diptera. The expanded OR repertoire, in combination with the reduced set of GRs might have evolved as a result of specialization on a single host plant and utilization of a long-range sexual pheromone. Possibly when life is short, long-range cues predominate, as there might simply be no time to land on potential hosts or mates and evaluate their quality upon contact.

Contributed talks III "Mixed session" Monday 25 June

Genome-wide association analysis for olfactory behavior in the *Drosophila melanogaster* genetic reference panel

Robert R H Anholt[1], Gunjan H Arya[2], Shilpa Swarup[3] and Trudy F C Mackay[4]

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Olfactory behavior is a quantitative trait determined by multiple segregating genes that are sensitive to the environment. The recently developed *Drosophila melanogaster* Genetic Reference Panel (DGRP) enables us to capitalize on natural variation to investigate the genetic basis of variation in olfactory behavior. The DGRP consists of 192 wild-derived inbred lines with sequenced genomes and identified sequence variants. Genetic variation among individuals within each line is minimal, whereas genetic variation among the lines is maintained and reflects the variation of the population from which they were derived. The lines are genetically variable for olfactory behavior, and phenotypic variation within the DGRP far exceeds that observed among *Drosophila* laboratory strains. We have measured olfactory responses of the DGRP lines to 14 odorants and identified 571 genes that harbor SNPs associated with phenotypic variation in responses to one or more odorants. Polymorphisms associated with variation in responses to different odorants do not necessarily reside in olfactory receptors, but cover a range of gene ontology categories, with overrepresentation of genes associated with functions of the nervous system, and 71 genes comprising a network centered on axonal path finding and neural connectivity. This unexpected finding suggests that, unlike physiological measurements of antennal sensilla, variation in behavioral responses to odorants depends on the perceptual integrated neural representations of chemosensory sensations, and variation in responses to different odorants accompanies variation in different molecular neural substrates. Analyses of segregating alleles in advanced intercross DGRP-derived populations and RNAi targeted gene knockdown can be used for functional validation of associated SNPs.

Symposium 13 "Plasticity and modulation in olfactory systems - Linnaeus Symposium" Monday 25 June

Experience-dependent plasticity in the chemosensory system of a moth

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[2]Angers University, Laboratoire Récepteurs et Canaux Ioniques Membranaires, Angers, France
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The effect of repeated exposure to sensory stimuli, with or without reward is well known to induce stimulus-specific modifications of behaviour in animals, described as different forms of learning. We found that even a single brief pre-exposure to different sensory stimuli can induce sensitization within or across modalities in noctuid moths. We observed intra-and cross-modal effects of brief pre-exposure to different stimuli on the behaviour of male moths and investigated underlying neural mechanisms. Males respond to low doses of sex pheromones to find a mating partner and to flower odours to find food sources. For feeding, the quality of a food source is evaluated upon contact, where sucrose concentration indicates nutritional value and bitter substances indicate non-palatability or toxicity. Male moths also detect ultrasound emitted by bats, their natural enemies. Behavioural analyses show that a brief exposure to any of the mentioned sensory stimuli increases responses to the sex pheromone after 24h. In addition, responses to gustatory stimuli are increased by brief pre-exposure to gustatory and pheromone stimuli. These reciprocal effects indicate that the phenomena described must represent a form of general sensitization or maturation of the sensory systems, rather than selective attention.

Attempts to localize modifications after brief pre-exposure within the nervous system indicate no or rather small changes in peripheral sensory systems. In the primary olfactory centre, the antennal lobe, neuron response thresholds to odours decreased after pre-exposure to the pheromone, to a bat-mimicking sound, but not to sucrose. Also the part of the antennal lobe processing pheromone information increased in size after pre-exposure, probably indicating an increase in synaptic connections. These first neurobiological approaches indicate that intra- and cross-modal effects of sensory pre-exposure might originate from changes at multiple levels within the nervous system.

Contributed talks I "Modulation of the olfactory system-Linneaus symposium" Monday 25 June

Olfactory sensory neurons plasticity induced by postnatal odorant exposure.

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Olfactory sensory neurons (OSNs) transform chemical information into electrical signals and send these signals to the brain, connecting it directly to the outside world. Little is known about the consequences of long term odorant exposure on OSNs. Here we report the molecular and physiological effects of odorant exposure at the cellular level. MOR23-GFP mice were exposed to Lyral, the ligand of the MOR23 receptor daily for 21 days starting at birth.

To study molecular changes within individual OSNs, mRNA levels for olfactory signaling pathway components were quantitatively analyzed using qPCR on GFP-containing neurons (6-8 per mouse). mRNAs for cyclic nucleotide-gated channel A2 subunit (CNGA2), calmodulin-stimulated phosphodiesterase (PDE1C) and MOR23 olfactory receptor were up-regulated by a factor of 3.7, 12.1 and 2.9 ($p<0.05$) respectively between control (n=5) and exposed group (n=5). Adenylyl cyclase 3 (AC3) transcript levels remained stable. This upregulation was not observed in analysis of whole OE tissue.

We then performed patch-clamp recordings on the dendritic knobs of MOR23 neurons in an intact preparation. MOR23 neurons responded to Lyral with inward currents with increasing amplitudes elicited by increasing concentrations. Dose-responses curves were fitted with Hill equation. Exposed OSNs displayed a lower detection threshold compared to control OSNs. The dynamic range of the dose-response was broader in exposed OSNs: their Hill coefficient was lower than in control neurons (n= 0,64 +/-0,06 vs 1,02+/-0,12, $p<0.05$). Responses of exposed neurons were also faster and shorter than the response of control neurons. At 10 µM, their rise-time was shorter (279ms+/-38, n=10) compared to control OSNs (396ms+/-4, n=12). The total current was reduced in exposed (183 pAs +/-35) compared to control OSNs

(366 pAs +/-62, $p<0.05$).

These observations suggest that postnatal odorant exposure induces molecular and physiological plasticity in individual MOR23 neurons.

Poster session I Poster #359

Suppression of taste receptor hTAS2Rs expression of diabetics and life-style

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Background: There increases diabetes mellitus and becomes severer problem in aging society. United Nations made the political declaration “the prevention and control of non-communicable diseases” at Sep. 2011. In Japan there are 22 million diabetes and high risk people. Japanese Ministry of Health thinks diabetes as one of most important five diseases to be conquered. Diabetes progresses silently and is a worsen factor.

Purpose: Taste is important for joy of life and used to be useful tools to judge foods maybe to manage nutrition intake. We try to make clear the expression of taste receptor hTAS2Rs of diabetics. By our prior study taste of diabetics changed. If we can detect the change of taste, that will be for primary and secondary prevention.

Methods: The subjects are 30 diabetes out-patients without any taste disorders and willing to be volunteers. They have type 2 diabetes except one with type 1. 86 people without any diseases were studied as control. Their age is about 20~90 yrs-old.

We developed new human taste evaluation methods that analyze hTAS2Rs by RT-PCR used scraping smear of the tongue. PCR products were measured using fluorescence micro-capillary electrophoresis. That had Japan Patent 4800212 and USA Patents 8,017,334 B2 in 2011.Family history was also studied. Food frequency questionnaire and Pittsburg Sleep Quality Index score were studied as lifestyles.

Results: The character of subjects was age; 63.1±14.2 yrs-old, onset age of diabetes; 51.2±13.2 yrs-old and period of having diabetes; 12.0±8.0 yrs. The expression number of taste receptor hTAS2Rs of diabetics was significantly suppressed to about 1.0 to 1.5 compared to 14.0 of same age of control, showed $p<0.01$ every ages. From our prior studies the expression increases by growing and varieties of foods taken. They got hTAS2R receptors 8.7±6.3 at 20 yrs-old, 14.0±3.7 at 40 to 50ies as maximum.

Conclusion: Diabetes people expressed below 1/10 receptors, significantly suppressed.

Poster session I Poster #179

Spatial organization of odorant receptor gene alleles within the nucleus of olfactory sensory neurons.

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Each olfactory sensory neuron transcribes only one allele from one gene chosen from a large gene family of odorant receptor genes. The mechanisms involved in this monogenic and monoallelic expression remain unclear. Different studies have indicated that regulation of gene expression may be affected by gene positioning in the cell nucleus. We therefore analyzed the nuclear organization of olfactory sensory neurons and asked whether the position of the two alleles of a given odorant receptor gene within the nucleus correlates with their transcriptional activity. We performed 3D immuno-

FISH to determine the position of the P2 odorant receptor gene alleles in relation to transcription factories, euchromatin, heterochromatin and chromosome territories. Our results show that olfactory sensory neurons have a particular nuclear organization and that, in 70% of the analyzed nuclei, one of the two P2 alleles is found associated with heterochromatic blocks.

Supported by FAPESP and CAPES.

Poster session I Poster #193

Robust encoding of concentration in the accessory olfactory system

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The accessory olfactory system (AOS) is specialized for detecting social cues, a major source of which is urine. Evidence suggests that the identities and amounts of specific compounds present in urine, such as sulfated steroids, may be used to communicate meaningful information. However, the detection of concentration may be confounded by environmental uncertainty: stimuli such as urine may evaporate or be diluted in the environment in ways that are not necessarily relevant, complicating detection of ligand amount. Therefore, it is useful for the AOS to detect levels of ligands in a way that is robust to such perturbations. A way to achieve a stable representation of concentration is through ratio encoding. While the absolute concentration of compounds will change following dilution or evaporation, the relative concentration of compounds will not. A plausible mechanism to compute ratios is through logarithmic encoding, based on the principle that $\log(X) - \log(Y) = \log(X/Y)$. This scenario requires both inhibitory circuitry, which is found in the accessory olfactory bulb, and encoding of log-concentration, a task that is complicated by receptor saturation. A way in which this may be achieved involves log-concentration encoding at the population level. This requires neurons to express a range of sensitivities to the same compounds and pooling of information across cells. Using multielectrode array recordings of vomeronasal sensory neurons, we investigated whether log-concentration of sulfated steroids can be encoded by populations of neurons. We found that while individual neurons followed first order kinetics with a range of binding affinities, populations of neurons could represent log-concentration. Population responses to mixtures could be used to reconstruct both log-concentration and stimulus identity. This provides evidence that that AOS is in theory capable of robustly encoding concentration information based on sensory neuron activity and anatomic features.

Poster session I Poster #239

Odor evoked autobiographical memories recruit less strategic and more affective processing brain areas than memories evoked by words

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Behavioral evidence indicates that odor evoked autobiographical memories (OEAMs) are older, more emotional, less thought of, and induce stronger time traveling characteristics than autobiographical memories (AMs) evoked by other modalities. The main aim of this study was to explore the neural underpinnings of AMs evoked by odors. Participants were screened for specific OEAMs and later presented with the odor memory cue and its verbal referent in an fMRI paradigm. As the same OEAM was retrieved across both cue formats (odor and word), potential cue dependent brain activations were investigated. The overall results showed that odor and word cued OEAMs activated regions typically associated with recollection of autobiographical information. However, relative word cuing, an odor cuing of OEAMs resulted in higher brain activity in regions associated with visual vividness and emotion, such as occipital gyrus, cuneus, precuneus, insula and anterior cingulate cortex. Although no odors were presented, a verbal cuing of the OEAMs

activated areas highly associated with olfactory perception and imagery (e.g., piriform and orbitofrontal cortex,). Furthermore, word but not odor cuing produced significant activity in the PFC. This difference may reflect that odor cued AMs entails a more direct and automatic retrieval, whereas verbally cued OEAMs produce a more strategic retrieval process. Finally, recollection of OEAMs from the1st vs the 2nd decade of life showed specific activation in the right OFC, whereas the 2nd vs 1st decade reflected a deactivation in the left inferior frontal gyrus. This pattern may reflect that over time, episodic odor representations shift from being perceptually based to become more semantically driven.

Poster session I Poster #175

Olfactory learning, odor discrimination ability, and long-term odor memory in Asian elephants

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The present study demonstrates that Asian elephants, *Elephas maximus*, can successfully be trained to cooperate in an olfactory discrimination test based on a food-rewarded two-alternative instrumental conditioning procedure. The animals learned the basic principle of the test within only 60 trials and readily mastered intramodal stimulus transfer tasks. Further, they were capable of distinguishing between structurally related odor stimuli such as members of homologous series of aliphatic substances and enantiomeric odor pairs. The elephants remembered the reward value of previously learned odor stimuli after 2, 4, 8, 16, and 52 weeks of recess without any signs of forgetting. The precision and consistency of the elephants' performance in tests of odor discrimination ability and long-term odor memory demonstrate the suitability of this method for assessing olfactory function in this proboscid species. An across-species comparison of several measures of olfactory learning capabilities such as speed of initial task acquisition and ability to master intramodal stimulus transfer tasks shows that Asian elephants are at least as good in their performance as mice, rats, and dogs, and clearly superior to nonhuman primates. The results support the notion that Asian elephants may use olfactory cues for social communication and food selection and that the sense of smell may play an important role in the control of their behavior.

Contributed talks I "Modulation of the olfactory system (Linnaeus Symposium)" Monday 25 June

Olfactory learning during human sleep

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Stimulus presentation during sleep can influence previously learned information, but whether entirely new information can be learned during sleep remains controversial. Olfaction may offer unique insight into this question because although odorants presented during sleep don't wake, they nevertheless drive a stimulus-specific sniff response that can be used as a measure of information processing and learning. To test the hypothesis that humans can learn new information during sleep, 34 subjects participated in a study where during sleep we generated partial trace conditioning between tones (either

1200 Hz or 400 Hz, duration = 1 sec, non-arousing < 40db) followed by (>1 sec ISI) odors (pleasant or unpleasant, duration = 3 sec, non-arousing olfactometer), and later measured the sniff-response following the tones alone. Consistent with stimulus-specific olfactory processing during sleep, subjects sniffed more vigorously following pleasant versus unpleasant odors during sleep (normalized volume: 0.96 ± 0.01 versus 0.90 ± 0.01, respectively, $F(1,27) = 13.7$, $p <$

0.001). Consistent with our hypothesis, during sleep subjects sniffed more vigorously to a tone alone previously paired during sleep with a pleasant odor, than to a tone alone previously paired during sleep with an unpleasant odor (normalized volume: 1.02 ± 0.01 versus 0.94 ± 0.01, respectively, $F(1,17) = 6.9$, $p < 0.02$). These newly acquired tone-induced sniff-modulations were retained in ensuing wake ((normalized volume: 0.96 ± 0.02 versus 0.89 ± 0.02, respectively, $F(1,27) = 5.04$, $p < 0.03$). In other words, subjects had acquired new learning such that in wake they sniffed to a tone, without ever knowing that tones and odors were paired in sleep. This finding suggests humans can learn new information during sleep, despite unawareness for the learning process, and for the learned information.

Poster session II Poster #82

Bioefficacy of utilized long-lasting insecticide treated nets (PermaNet®2.0) against malaria vector mosquito

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The study was conducted to evaluate the bioefficacy of long-lasting insecticide treated nets (PermaNet®2.0) over time, in relation to the species composition of *Anopheles* mosquitoes and malaria transmission in rural villages around Bahir Dar, Ethiopia. The space spray collection method was used to determine the species composition of indoor resting *Anopheles* mosquitoes and to identify the major malaria vector in the study area. The field collected samples of household used PermaNet® 2.0 long-lasting insecticide treated nets (LLITNs) were tested for their bioefficacy against laboratory reared *An. arabiensis* using the World Health Organisation standard cone test protocol.

The study revealed that 75% of *Anopheles* mosquitoes collected from indoor location were *An. arabiensis*, indicating that this species was the major vector of malaria in the study area. The mean percentage knockdown effect of PermaNet® 2.0 LLITNs after both six months and two years of household usage against females *An. arabiensis* was 100%. However, this effect decreased to 44.5% after three and half years of household use. There was no significant difference ($P > 0.5$) in the mean percent mortality caused by PermaNet®2.0 LLITNs after six months and two years household usage (92.5% and 84%, respectively). However, there was a markedly significant reduction ($P < 0.01$) in the mean percent mortality of females *An. arabiensis* exposed to PermaNet® 2.0 LLITNs with three and half years of household usage (27%) against under laboratory conditions.

The PermaNet® 2.0 LLITNs distributed in the study area remained highly effective for up to two years of constant household use. However, the efficacy of the nets declined to a very low level after three and half years of household utilization. Therefore, bed net distribution activities should be accompanied by continuous follow up studies on the bioefficacy of long-lasting insecticide treated nets within every six months household utilization period to determine the exact replenishment period. Furthermore, detailed entomological studies on the species composition in different seasons and the behavior of *Anopheles* mosquitoes in relation to insecticide treated nets utilization and indoor residual spraying need to be conducted.

Poster session I Poster #83

Study on the performance of lures and traps for the management of the sorghum chafer, *Pachnoda interrupta* in Northern and Eastern Ethiopia

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The Sorghum chafer is a devastating insect pest for agriculture in general and especially for sorghum and maize, particularly in Northern and Eastern Ethiopia. Semiochemicals identified from the insect itself and host plants could be potential agents for selective detection and monitoring of the sorghum chafer. The beetle has an aggregation pheromone and host odors for attraction. Previous studies on the basic electrophysiology and behavior have laid the foundation for the current ongoing study. The most important compounds were phenylacetaldehyde, 2,3-butanediol, methylsalicylate eugenol and isoamylacetate. Attractiveness of those volatile compounds can be improved with appropriate dispensers using 4 ml vials and cotton wick inside to release the odor in optimum amounts in the field.

The cumulative trap catch over the field experiments was used for analysis using ANOVA. This field based with complete randomized block design, N=10, experiment has shown promising results in the performance of both traps and lures along with dispensers. The results have shown that locally available and inexpensive traps (the green bucket) developed from locally available materials could replace those commercially available but unaffordable traps (the Japanese beetle trap, for instance) with further modifications. For more elaboration, the field based results which we believe would evoke further discussions will be presented.

Poster session II Poster #296

Disease systems chemical biology of flavors.

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Although, the human olfactory system consists of around 350 odorant receptors (ORs) with diverse sensitivity to flavor molecules, the human odor perception and how odorants might play a major role in our systems biology remain largely unknown.

Here, we present a global mapping of flavor molecules in the pharmacological space. Based on a chemogenomic database called ChemProt[1], we developed an odorant-target matrix to explore the relationships between chemical structures, biological targets and diseases susceptibility. To validate our approach, we tested seven compounds for the peroxisome proliferator-activated receptor gamma (PPARg). Six showed PPARg agonist activities, suggesting potential therapeutic effect for diabetes and inflammation.

In a second step, we explored the complexity of the OR-flavor relationships in human defined as odorome. We developed a protein-protein association network (OR-OR) in order to identify potential novel OR-flavor relationships not yet annotated. The OR-OR network is generated under the assumption that if two proteins are affected with two chemicals, then both proteins are deemed associating in chemical space[2]. The developed human odorome will help to understand the underlying molecular mechanisms of flavors and the biological pathways they perturb by integrating protein-protein interaction data, protein-disease annotations and functional annotation of proteins.

With the proposed computational systems biology approach, identification of disease-gene associations within the human odorome are of potential interest, especially with the fact that many neuropsychiatric disorders might be accompanied by a decrease or increase in odor detection.

1. *Nucleic Acids Res.* **2011** Jan;39 (Database issue):D367-72
2. *PLoS Comput. Biol.* **2010** May20;6(5):e1000788

Poster session II Poster #422

Biogenic amines activate human blood leukocytes via trace amine-associated receptors (TAAR)

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Biogenic amines are chemical messengers that act as endogenous hormones, neurotransmitters, or neuromodulators on specific receptors. Certain biogenic amines (e.g. 2-phenylethylamine (2-PEA), tyramine, 3-iodothyronamine (T1AM)), which are present at nanomolar concentrations in the blood and throughout the central nervous system, specifically interact with 'trace amine-associated receptor 1' (TAAR1) – a G protein-coupled receptor – which was shown to modulate brain monoaminergic systems. Other TAAR are also expressed in neurons of olfactory epithelia of mouse and zebrafish, where they may detect amines as odorants or pheromones. However, while TAAR1, the most ancient of all extant TAAR, is absent from olfactory epithelia, its mRNA was observed in a variety of other peripheral tissues, including stimulated human leukocytes, suggesting an ancestral and so far unknown, non-olfactory function of TAAR1.

Here we show the presence of gene transcripts for five out of six human TAAR in blood leukocytes. We found that TAAR1, and its closest relative TAAR2, mediate main cell functions, such as chemotaxis, interleukin-4 and immunoglobulin E secretion, in isolated TAAR-positive neutrophils, T- and B-lymphocytes, respectively, in the presence of nanomolar concentrations of their ligands 2-PEA, tyramine, and T1AM. These leukocyte functions were largely abolished by siRNA knock-down of TAAR expression. In T- and B-lymphocytes, mRNA hybridization experiments revealed partially overlapping subsets of TAAR1- and TAAR2- expressing cells, suggesting TAAR as activation markers to define discrete functional subsets of lymphocytes.

Thus, our data demonstrate that biogenic amines, which can be found in certain foods at high concentrations, have a

direct role on our cellular immune system, and thus put TAAR signaling in leukocytes into the focus of amine intolerance and food safety.

Poster session II Poster #226

Untypical connectivity from olfactory sensory neurons expressing OR37 into higher brain centers visualized by genetic tracing

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The OR37 subfamily of odorant receptors (OR) exists exclusively in mammals. In contrast to ORs in general, they are highly conserved within and across species. These unique features raise the question, whether olfactory information gathered by the OR37 sensory cells is processed in specially designated brain areas. To elucidate the wiring of projection neurons from OR37 glomeruli into higher brain areas, tracing experiments were performed. The application of DiI onto the ventral area of the olfactory bulb, which harbors the OR37 glomeruli, led to the labeling of fibers not only in the typical olfactory cortical regions, but also in the medial amygdala and the hypothalamus. To visualize the projections from a defined OR37 glomerulus more precisely, transgenic mice were studied in which olfactory sensory neurons co- express the receptor subtype OR37C and the transsynaptic tracer Wheat Germ Agglutinin (WGA). WGA became visible not only in the OR37C sensory neurons and the corresponding OR37C glomerulus, but also in cell somata located in the mitral/tufted cell layer adjacent to the OR37C glomerulus, indicating a transfer of WGA onto projection neurons. In the brain, WGA immunoreactivity was not detectable in typical olfactory cortical areas, but instead in distinct areas of the medial amygdala. Detailed mapping revealed that the WGA immunoreactivity was restricted to the posterior-dorsal subnucleus of the medial amygdala. In addition, WGA immunoreactivity was visible in some well circumscribed areas of the hypothalamus. These results are indicative for a unique connectivity from OR37C sensory cells into higher brain centers.

This work was supported by the Deutsche Forschungsgemeinschaft

Contributed talks VI "Interactions" Monday 25 June

Attraction of female *Anopheles stephensi* mosquitoes to the spores of entomopathogenic and non-entomopathogenic fungi

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We performed behavioral Y-tube olfactometer assays in the laboratory to test the upwind flight responses of the mosquito, *Anopheles stephensi* a major vector of human malaria in Asia, toward the fungal spores of two entomopathogenic species, *Beauveria bassiana* and *Metarhizium acridum*. Our group has been working toward the goal of applying spores to various indoor surfaces of dwellings in order to cause mosquitoes to land on and pick up spores to induce mosquito infection and mortality. *B. bassiana* is known to kill infected mosquitoes within 6-8 days after contact with spores. In one project, the spores' nascent attractiveness or repellency first needed to be determined in order to decide whether or they needed to be augmented with synthetic attractants to induce landing and infection. Unfed female An. stephensi mosquitoes were tested one-at-a-time for their upwind flight propensity toward the arm of the Y-tube that contained a 50 mg loading of spores, versus the other arm that contained a blank filter paper. The females clearly exhibited a strong attraction towards *B. bassiana* spores; 75% of the females tested flew upwind to within 1 cm of the spores, and a significant percentage of these actually landed on the spores themselves. A high percentage of the mosquitoes (70%) was likewise attracted to 50 mg of the spores of *M. acridum*, and also to a lesser degree (65%) to 50 mg of the spores of *Aspergilus spp*., a non-entomopathogenic species. The spores of *Penicilium spp*., on the other hand, were deterrent to An. stephensi females, 80% of which preferred to fly up the blank arm of the Y-tube olfactometer and not to the *Penicilium* spores. In competitive choice-tests between the spores of different fungal species, those of *B. bassiana*

were preferred, with 67%, 67% and 82% flying upwind to these spores vs. those of *M. acridum*, *Aspergillus spp*. and *Penicillium spp*. , respectively.

Symposium 14 "Higher olfactory processing - Delwart Symposium" Tuesday 26 June

The impact of neuronal diversity and neural activity on olfactory processing circuits.

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The sense of smell is tasked with recognizing distinct combinations of molecularly diverse odor molecules and linking them to innate and learned behavioral outputs. Odor components are detected by a diverse set of olfactory sensory neurons in the nose and neural circuits in the olfactory bulb process this information. The mitral and tufted (MT) neurons transmit sensory information from the olfactory bulb to higher cortical centers where integration of signals from different classes of MT neurons occurs. In contrast with other sensory systems, the developmental mechanisms that control the formation of higher olfactory circuits are poorly understood. We have developed a multi-color long-range viral labeling method to trace small groups of individual MT neurons and map their axonal projections using three dimensional brain reconstructions. We previously reported that "sister" MT neurons that carry related sensory information exhibit extensive diversity in axon targeting, even within the same animal. These results suggest that cortical olfactory neuronal circuits may arise through stochastic patterning mechanisms, which could be impacted by neural activity or intrinsic genetic programs. We have exploited our single neuron tracing system to address the effect of neural activity and neural cell type on the formation of distinct olfactory circuits in the olfactory bulb and olfactory cortex. Our results show that cell type and neural activity impact neuronal circuit formation differently in different axonal targets of the same neuron and provide a toolkit to visualize and compare the morphologies of projection neurons that innervate broad complex cortical regions.

Poster session I Poster #95

Controlling the multisensory world of an insect during functional imaging

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We present a novel fully automated technique that allows for the detailed temporal control of visual, olfactory and taste stimulation during *in vivo* calcium imaging of the insect brain. The set-up makes it possible to record changing brain activity as the animals learn. All devices, including the microscope, are controlled from a single computer to allow the relative timing of the signals and measurements to be manipulated.

Visual stimuli are generated by LEDs with different spectral characteristics using a custom made driver unit that controls their intensities. Optically isolated fiber-optic light guides are used to transfer the visual stimuli to the eyes of the insect. This arrangement allows the eyes to be stimulated without interfering with the functional imaging of the brain activity. For odour stimulation, antennae are ventilated from a glass tube with a continuous charcoal-filtered and moistened air stream. During odour stimulation, the air stream is switched from an empty pipette to an odour-laden one to minimise the influence of added air volume. Taste stimuli are applied using a servo-controlled micro-actuator that moves a small container towards the tip of the proboscis of the animal.

To analyse the signals, the recorded images are first spatially filtered using a Gaussian filter to remove noise. This is followed by an estimation of $\Delta F/F$ for each frame, where F is estimated using a sum of exponential functions fitted to the parts of the calcium fluorescence decay curve outside the potential response. The response magnitude is calculated as the average $\Delta F/F$. Latency and duration of the signal can also be established.

Non-parametric statistical test are used to compare activity levels and principal component analysis together with linear discriminant analysis is used to establish patterns in the brain activity.

Plenary lecture Sunday 24 June

Genes, connectomes, and decisions: using fixed circuits to drive flexible behaviors

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How do genes and the environment interact to generate a variety of behaviors? How are behavioral decisions modified by context and experience? Genetic variation, internal states, and environmental influences converge on common, anatomically-defined neuronal circuits to regulate behaviors in the nematode worm Caenorhabditis elegans. Analysis of these circuits shows the detailed wiring diagram of C. elegans is both incomplete and ambiguous, because information processing can be altered by modulatory inputs that are invisible in the anatomical wiring. These modulatory influences shape an apparently "overconnected" nervous system to select appropriate behaviors from a larger number of alternative, latent circuits.

Symposium 22 "Odor memory and perception: cells to circuits" Wednesday 27 June

Mechanisms underlying high-skill olfactory learning-induced enhancement of synaptic transmission

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Training rats in a particularly difficult olfactory-discrimination task results with acquisition of the skill to perform superbly in this very complex task. Such skill acquisition, termed 'rule learning' or 'learning set, is accompanied by a series of pre and post–synaptic cellular modifications in layer II pyramidal neurons of the piriform cortex (**PC**). Long-term enhancement occurs in the three components controlling neuronal activation; the excitatory synaptic drive mainly mediated by glutamate receptors, the intrinsic neuronal excitability, and synaptic inhibition mediated by $GABA_A$ and $GABA_B$ receptors .These modifications have two major common traits:

a. They are widespread throughout the piriform cortex network. Both physiological and morphological modifications are found in most of the pyramidal neurons in the netwrok

b. The time course in which these modifications appear and disappear is strongly correlated with the time course in which the skill is acquired and decays. However, memories for specific odors outlast these modifications by far. Thus, the identified modifications are related to rule learning, rather than to long-term memory for the specific odors for which the rats are trained.

Here we will describe how excitatory and inhibitory synaptic transmission are modified by complex learning, in a manner that supports long-term term memory of elaborated performance capabilities while maintaining the delicate balance between excitation and inhibition in the cortical network thus allowing stability of activation in the piriform cortex.

Symposium 21 "Molecular and neural basis of taste detection" Wednesday 27 June

Visualizing the Neural Representation of Taste at the Periphery: Imaging Ganglion Function In Vivo

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The perception of taste requires the propagation of tastant information from peripheral cells to the brain. Distinct populations of taste receptor cells on the tongue have been shown to encode each of the basic taste qualities. We have now developed a novel preparation to explore the representation of taste qualities in the first neural station *in vivo* (taste ganglia) using a combination of two-photon imaging, micro-endoscopy, and engineered mouse lines expressing genetically encoded calcium sensors. We will present the results of these studies.

Poster session I Poster #3

Morphological characterization of the antennal lobe output in *Drosophila*

Amelie Baschwitz[1], Antonia Strutz[1], Veit Grabe[1], Bill S Hansson[1] and Silke Sachse[1]

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In the fruit fly *Drosophila melanogaster* – just like other insects - the olfactory system is important to find food sources, good mating partners as well as oviposition sites and to avoid danger. Olfactory sensory neurons (OSNs), housed in sensilla on the antennae, express odorant receptors (ORs). The binding of an odor molecule to a specific OR evokes neuronal activity, which is transferred via the OSNs to distinct brain structures, so-called glomeruli, of the antennal lobe (AL), the first olfactory processing center in the fly brain. Within the glomeruli synaptic connections to second order neurons (projection neurons, PNs) as well as interconnections by local interneurons (LNs) take place. The latter is assumed to modulate the input signal via excitation and/or inhibition on particular synaptic terminals to OSNs and PNs. The modulated input signal is transferred via PNs to higher brain centers, like the mushroom body (MB) or the lateral horn (LH), leading to odor-guided behavior. For further anatomical characterization of the innervation pattern in higher brain centers of inhibitory and excitatory PNs we used photoactivatable GFP (PA-GFP). This method enables labeling of single neurons by irradiation of single somata or labeling of all PNs innervating a specific glomerulus. Of special interest are PNs innervating glomeruli that are predominantly activated by odors that elicit either aversive or attractive behavior. This study is supported by the Max Planck Society, the BMBF and the IMPRS.

Poster session I Poster #225

Mammalian specific OR37 receptors are differentially activated by distinct odorous fatty aldehydes

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The capacity of the mammalian olfactory system to detect an enormous collection of different chemical compounds is based on a large repertoire of odorant receptors (ORs). A small group of these ORs, the OR37 family, is unique due to a variety of special features. Members of this subfamily are exclusively found in mammals, they share a high degree of sequence homology and are highly conserved during evolution. It is still elusive which odorants may activate these atypical receptors. We have reasoned that compounds from skin, hairs or skin glands might be potential candidates. We

have exposed mice to such compounds and monitored activation of glomeruli through the expression of the activity marker c-fos in juxtaglomerular cells surrounding ventrally positioned glomeruli in the olfactory bulb (OB). Employing this methodology it was found that stimulation with long-chain alkanes elicits activation in the ventral part of the OB, however, none of the OR37 glomeruli. Analyses of long-chain hydrocarbon compounds with different functional groups revealed that long-chain aliphatic aldehydes elicited an activation of defined OR37 glomeruli, each of them responding preferentially to an aldehyde with different chain length. These results indicate that OR37 receptors may be tuned to distinct fatty aldehydes with a significant degree of ligand specificity.

This work was supported by the Deutsche Forschungsgemeinschaft.

Symposium 19 "Preference for umami taste controlled by chemical senses - Ajinomoto Symposium" Tuesday 26 June

Flavor perception, sensitive periods and infant growth

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Many of the most common human health problems (e.g. obesity, diabetes, hypertension, heart disease, some cancers) are related to the amounts and kinds of food eaten. Since the flavor senses play a central role in determining food choice, an understanding of how flavor compounds influence human food choice and modulate intake is critical if we are to develop strategies to diagnose, treat and ultimately prevent these nutritionally related diseases. Of particular interest is the ontogeny of responses to flavors since early exposure and experiences can have long-term, perhaps permanent, influences on later health. To develop a broader understanding of how early flavor experiences can influence food selection, intake and nutritional status, we have been studying flavor learning in infants who are fed formulas containing hydrolyzed casein protein (PHF) as their amino acid source. Unlike conventional cow milk formulas (CMF), PHFs are very high in free amino acid content. To adults the flavor of these formulas is extremely unpalatable but very young infants accept them readily. We have identified a sensitive period when exposure to these formulas renders them highly palatable - possibly for the rest of their lives. We recently found that infants fed PHF formulas grow at a rate that resembles that for the breast fed infant whereas infants fed CMF grow faster and, according to some studies, are more prone to later obesity. One explanation for differential growth is that PHFs are more satiating than CMFs, perhaps due to their large concentrations of free amino acids including the umami taste stimulus, glutamate. Since some evidence suggests that glutamate might play a role in satiety, we tested this hypothesis in short term, single feeding studies. Results supporting this hypothesis will be described.

Poster session II Poster #4

Yeast links *Drosophila melanogaster* with fruit

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Drosophila flies and other insects use fruit as substrate for offspring development. Sugar-rich fruit is also substrate for microorganisms like yeasts, which modify the food quality for fruit-associated insects. Odour, as an important sensory quality of food, is one factor that is changed by microbial processing. Fermentation for example might cause the production of attractive odours or in contrast produce off-flavours.

Studying attraction, feeding, mating and oviposition in *Drosophila melanogaster*, we found a strong effect of yeast on these different behaviours. Wind tunnel attraction behaviour was studied in more detail for different yeasts to identify potential behaviourally relevant attractants or off-flavours. In addition we studied the attraction to yeast in relation to the sex and mating state of *D. melanogaster*.

Evidently, yeast is not only a limiting factor in the larval diet, but also mediates adult behaviour that enables the

exploitation of fruit as food source. Our results demonstrate the primary importance of yeast for the physiology and ecology of *D. melanogaster* within the context of natural fruit yeast habitats. We want to point out that yeasts and other microorganisms occupy a commonly overlooked trophic level between plant and insect herbivore, which has to be considered, especially in ecological research on fruit-associated insects.

Poster session II Poster #280

Decrease of olfactory sensitivity during normobaric hypoxia

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The phenomenon altitude sickness is a pathological condition in humans caused by high altitudes where we find a low partial pressure of oxygen. Symptoms of this condition usually occur above 2,400 meters altitude and include disturbances of the sense of smell and taste. The aim of this research was to evaluate olfactory and gustatory abilities of healthy subjects during baseline conditions and after seven hours of normobaric hypoxia. Also, we aimed to determine the relation of behavioral measures and volume of different brain structures. Therefore, olfactory function 16 healthy, normosmic subjects was assessed using the Sniffin' Sticks discrimination and n-butanol threshold test, as well as intensity and pleasantness ratings. Gustatory function was evaluated utilizing the Taste Strips. Each of the aforementioned tests was conducted twice, firstly under baseline conditions (21% O2, 78% N2, 21-23°C, 30-50% relative humidity, equivalent to conditions at app. 518 meters altitude) and secondly after seven hours of normobaric hypoxia exposure (13% O2, 86% N2, 21-23°C, 30-50% relative humidity, equivalent to conditions at app. 4000 meters altitude). At the end of each testing session, structural MRI scans were acquired at a 3T clinical MRI scanner. Evaluation of the imaging data was accomplished with the help of Matlab, SPM8 and the VBM8 toolbox. During normobaric hypoxia a significant reduction of olfactory sensitivity as well as intensity estimates was established. Olfactory discrimination and gustatory function was not influenced by hypoxic conditions. Volumetric imaging data showed a significant positive correlation of gray matter in brain areas typically related to olfactory processing with olfactory threshold values. We conclude that normobaric hypoxia leads to a significant decrease of olfactory sensitivity and intensity evaluation. These impairments can be explained by the smaller volume of typical olfactory brain areas.

Symposium 16 "Taste and beyond - integration of nutrient sensor functions in oral cavity and gut - Ajinomoto Symposium" Tuesday 26 June

Nutrient sensing in the gut: physiological implications in the control of food intake

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Intestinal chemosensitivity is a key element in digestive processes. One of the first physiologists who proposed a chemosensitivity hypothesis was Pavlov in the 19th century. Based on his 'nervism', i.e. the theory that the 'nervous system controls the greatest possible number of bodily activities', he suggested that sensory nerves are exposed to the intestinal lumen and directly catch chemical messages of the luminal content at their nerve endings. Bayliss and Starling in 1902 observed that the presence of protons in the proximal small intestine elicited a strong stimulation of pancreatic- fluid secretion. They named the secreted compound 'secretin' and used Hardy's term 'hormone' as a general designation for blood-borne chemical messengers. To date, intestinal chemosensitivity is thought to involve a highly specialized and complex system of primary afferent neurons, intestinal chemosensory cells and the gut immune system controlling digestive processes including food intake.

Entero-endocrine cells (EEC) are able to *'taste'* the luminal content and to function as chemosensory transducers to provide the interface between the intestinal lumen and the afferent nerve terminals. Their secretory products - mainly peptide hormones such as cholecystokinin, GLP-1 and peptideYY - are released upon stimulation by nutrients into the extracellular space of the lamina propria to either act 1) locally in a paracrine fashion to activate afferent terminals, or other cells, or 2) in an endocrine fashion via intestinal capillaries to bind to specific receptors at more distant targets.

The chemosensory mechanisms by which EEC sense the intestinal lumen remain poorly understood. Recent information suggest that taste signaling mechanisms known from the oral epithelium also operate in the mucosal epithelium. Several nutrient-responsive G-protein coupled receptors (GPCRs) have been identified in EEC (sweet-taste receptor, GPR120 responsive to free fatty acids (FFAs). This review will provide a brief overview on gastrointestinal chemosensory mechanisms and their functional involvement in the secretion of satiation peptides with a focus on human studies.

Poster session I Poster #317

Bitter taste receptors in the gut

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Bitter taste fulfills an important role in the quality assessment of food. Numerous natural bitter substances are toxic and hence, their consumption may be fatal. Since oral recognition of compounds by bitter taste receptors (Tas2rs) is generally linked to rejection behavior, the primary role of bitter taste is believed to protect organisms from intoxication.

Outside the oral cavity Tas2rs were also detected in respiratory and gastrointestinal (GI) tissues. In respiratory epithelia it is assumed that Tas2rs have, analogous to the gustatory system, protective functions. Within the GI tract, however, Tas2r activation has been associated with a variety of physiological effects. Not only the exact role of GI-resident Tas2rs is rather elusive, also their cellular expression pattern is largely unknown.

To investigate whether Tas2rs are indeed expressed in GI tissues and to determine their role within the gut, we performed experiments using GI tract-derived cell lines and mouse tissues. By RT-PCR analyses and functional experiments using a battery of bitter compounds we investigated if NCI-H716 cells, a model of human enteroendocrine cells, express TAS2Rs and if their expression correlates with functional responses. In the GI tract of mice we studied the expression of Tas2rs by RT-PCR and histological experiments.

Analyses of NCI-H716 cells revealed that TAS2Rs and taste-related signaling components are present, however, the correlation between receptor expression and function is limited. Studies in mouse tissues demonstrated the expression of Tas2r genes by RT-PCR. However, further experiments trying to localize Tas2r expressing cells were not successful. Extending our analyses to genetically modified mice, which express CRE recombinase from the locus of the bitter receptor Tas2r131 to drive the expression of tdRFP, we finally noticed fluorescent cells. Subsequent co-localization experiments with cell type-specific markers allowed the characterization of these cells.

Poster session II Poster #84

An ancient olfactory acid/base sensor in insects

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Embedded within the antennal olfactory organ of *Drosophila melanogaster* is an unusual sensory structure called the sacculus. The sacculus is comprised of three distinct chambers, each lined with sensilla housing 2-3 neurons. Previous morphological, anatomical and surgical studies of sacculus neurons have implicated them in chemosensation, hygrosensation and/or thermosensation. While a subset of sacculus neurons have been shown to play a role in temperature detection (Gallio et al. Cell 2011), the function of this organ has remained largely mysterious, due to its

inaccessibility to peripheral electrophysiological analysis.

We have recently shown that sacculus neurons express members of the Ionotropic Receptor (IR) family of chemosensory receptors (Benton et al., Cell 2009). Promoters of these IR genes were used to drive expression of anatomical (CD8:GFP) and physiological (G-CaMP) reporters in subpopulations of sacculus neurons, to permit their morphological and functional characterisation. Neurons in sacculus chambers I and II express IR40a+IR93a together with the co-receptor IR25a, and respond to amine and other basic odours. Neurons in chamber III express IR64a with its co-receptor IR8a, and respond principally to acidic odours (Ai et al., Nature 2010). Notably, we observe subpopulations of IR40a and IR64a neurons display heterogeneous responses to their respective ligands, strongly implying the existence of other factors that define their functional properties. Comparative genomic analysis of these IRs across insects reveals them to be among the most conserved of this receptor repertoire, suggesting that the sacculus represents an evolutionarily ancient insect olfactory acid-base sensor.

Poster session II Poster #88

Isolation of cues that drive mosquito preference for certain human hosts

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More than 400 million people are infected annually by mosquito-vectored diseases such as malaria, Dengue fever and West Nile fever. Understanding how mosquitoes select a human host is therefore an important global health concern. It has long been noted that while many mosquito species are strongly attracted to humans, they are not equally attracted to all human hosts. We are interested in understanding the cues that female mosquitoes use to choose a particular human host. Towards this goal, we have developed a uniport olfactometer as a method for assessing the attraction of mosquitoes to human subjects, expressed as an individual's "attraction index." We have found that subjects differ significantly in their attraction index, and that these differences are stable over time. Critically, we have found that differences in attraction index cannot be fully explained by differences in body temperature or skin surface area. Thus, we believe that differences between subjects may largely be mediated by differences in body odor. Skin microbiota are a major source of the volatiles that constitute human body odor, and the diversity of bacteria present on human skin varies significantly between individuals, likely reflecting aspects of their internal physiology. We hypothesize that bacterially-produced volatile cues may communicate information to female mosquitoes about the blood nutrient value of a potential human host and thus might influence host selection. We are currently conducting a pilot screen of 20 human volunteers to determine how attractive they are to mosquitoes. In parallel, we are collecting blood and skin microbiota samples from these individuals to ask if there is a relationship between the composition of a subject's skin microbiota or blood metabolites and attractiveness to mosquitoes. The pilot data will allow us to launch a larger study, aimed at pinpointing the sensory information mosquitoes use to discriminate between hosts.

Symposium 11 "The stimulus – odor space and chemometrics" Sunday 24 June

Complexity of odorant structure influences perception and olfactory cortex activity

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An important issue in olfaction research is to relate percepts to the molecular structure of stimuli. Previous studies attempted to relate odor quality to the odorant's physicochemical parameters. Here, we evidence from psychophysics studies a quantitative structure-odor relationship in which the more structurally complex a monomolecular odorant, the more numerous the olfactory notes it evokes. In a second neuroimaging study we examined how such quantitative structure/percept relationship was reflected in the human olfactory system. To this end, 20 human participants were stimulated with two low complexity odorants (guaiacol and isoamyl acetate) and two high complexity odorants (R-

limonene and terpinenol). Responses were assessed by fMRI (1.5T -Siemens Sonata). Stimuli were delivered to the subjects using an air-dilution olfactometer; after the functional scans, participants were to estimate intensity, pleasantness, familiarity of the stimuli and the number of olfactory notes. Results showed that whereas the same intensity, pleasantness and familiarity ($p>.05$ in all cases) were judged for low and high complexity odorants, the latter induced more olfactory notes than the former ($p=.015$). A preliminary analysis performed on the imaging data using SPM8 revealed differential piriform activation as a function of odorant molecular complexity. These findings suggest that complexity of odorant molecules provides a framework to explain both the subjective experience of smells and its neural processing in the olfactory cortex.

Symposium 23 "Evolution of chemosensory systems " Wednesday 27 June

Olfactory evolution in *Drosophila* (EMBO Young Investigator Lecture)

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The detection of odours in the environment is universally important for primal behaviours such as feeding, mating, kin interactions and escape responses. Moreover, animal olfactory systems display enormous evolutionary capacity, as species acquire and discard olfactory receptor genes, neurons and behaviours in an ever-changing landscape of external chemical stimuli. I will present our recent insights into olfactory system evolution yielded by analysis of a recently-discovered family of olfactory receptors, the Ionotropic Receptors (IRs), and their neuronal circuits, in *Drosophila*.

Poster session I Poster #297

Neuroimaging of olfaction in obsessive-compulsive disorder

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Background: Obsessive-compulsive disorder (OCD) is a common psychiatric illness. Neuroimaging studies show that compared to healthy controls (HCs), OCD patients have greater activation in their right insula to disgusting images. OCD patients may in fact be more sensitive to unpleasant stimuli regardless of the sensory modality, which may trigger their subsequent obsessions and compulsions. Therefore, we investigated the function of the olfactory system in response to pleasant and unpleasant odors in OCD patients compared to HCs using functional magnetic resonance imaging (fMRI). **Methods**: 7 OCD subjects and 8 matched HCs were exposed to pleasant (banana, vanilla, chocolate) and unpleasant (garbage, feces, urine) odors using our specially developed olfactometer during fMRI. Unscented air was the control stimulus. Subjects rated stimuli on intensity and valence and completed the UPSIT and a set of questionnaires measuring OCD symptoms, disgust sensitivity, and emotion. **Results**: Compared to HCs, in response to unpleasant (vs. pleasant) odors OCD patients had increased activation in their right anterior insular, left posterior insular, and anterior cingulate cortex/superior cingulate; and decreased activation of their left lateral orbitofrontal cortex, left dorsolateral prefrontal cortex, and putamen (bilateral). **Conclusion:** Similar to the results in the visual domain, people with OCD appear to be more "neurally sensitive" to unpleasant odors. Their decreased activation in prefrontal regions in response to unpleasant odors implies that they have less cognitive/top-down control over their increased unpleasant feelings (indicated by increased insula and cingulate activation). This is the first study to examine olfaction in OCD using fMRI and further elucidates the neural underpinnings of OCD, which may contribute to the development of better methods of treatment. These results warrant further studies with larger sample sizes and other anxiety disorder comparison groups.

Symposium 8 "Central mechanisms of taste learning and memory" Sunday 24 June

Reactivation of Glutamate and Catecholamines in either insular cortex and amygdala are involved on taste aversion memory consolidation.

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It has been considered for some time that memory consolidation requires post-trial stabilization of synaptic information storage. In this regard, it has been speculated that waves of receptors activation, immediate-early genes and replenishment of receptor subunit pools by the synthesis of new proteins occur to induce functional or morphological changes to maintain the information for longer periods. In this paper, we are providing evidence that post-acquisition re-activation of two important neurotransmitter systems the catecholamines and the glutamate are involved in taste memory consolidation. We have used in vivo microdialysis attached to a capillary electrophoresis system to quantify the simultaneous release of glutamate and dopamine/norepinephrine in either the insular cortex or amygdala during an associative taste learning task and 90 min after acquisition. In this learning task, animals associate a novel taste stimulus with a gastric malaise separated by several minutes, which allows to determine accurately the molecular events associated with the CS and U.S presentations. The results showed clear post-acquisition re-activation increments of glutamate and dopamine/norepinephrine 45 minutes after the CS-US association. These post-acquisition re-activation increments did not appear in backward conditioned control groups that were unable to acquire the task. Blockade of a combined D1 and/or NMDA receptors in the insular cortex, or NMDA and/or β-adrenergic receptors in the amygdala before the off-line activity impaired long- but not short-term memory. To determine whether the reactivation of these molecules are indeed functional, we decided to do two infusions into the amygdala of both NMDA and isoproterenol (a β-adrenergic agonist) after the CS exposure, in order to emulate a CTA without the US presentation. These combined injections induced a clear taste aversion, whereas similar infusions of isoproterenol or NMDA induced only milder effects. These results suggest that post-aquisition release of glutamate and catecholamines have an important functional role in memory consolidation of taste aversion. Supported by: CONACYT 060478.

Poster session II Poster #106

Olfactory evolution in cave-dwelling *Astyanax mexicanus* fish populations

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While the general features of the olfactory system are well-conserved across vertebrates, olfactive capabilities have been known to evolve rapidly in response to changing environmental conditions. In cave-dwelling populations of the fish *Astyanax mexicanus*, which have evolved from surface-dwelling ancestors, several morphological and behavioral shifts occurred in adaptation to cave life characterized by total and permanent darkness. Previous studies have suggested that sensory systems such as the lateral line (to navigate in the dark) or the olfactory system (to locate food and mating partners) are modified in cavefish. Notably, the early embryonic development of the forebrain is modified, resulting in increased proliferation in the ventral telencephalon and enlarged olfactory bulbs. Current research efforts are focused on investigating the cellular and molecular mechanisms underlying possible olfactory adaptations in *Astyanax* cavefish populations. We extend our analysis of telencephalic neurogenesis and the signaling pathways that control the production of olfactory interneurons to study the contribution of modified neurogenesis on olfaction in cave-dwelling populations. Utilizing recently-acquired 454 transcriptome sequencing data, we also compare the expression of olfactory receptor genes in cave- and surface-dwelling populations to study the potential contributions of olfactory receptor repertoire evolution on adaptation in cavefish populations. Finally, behavioral analyses are currently being implemented in order to link olfactory system modifications to the behaviors that they control.

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Poster session II Poster #278

Chemosignal evaluation in the amygdala: Possible function of medial amygdala and medial-caudal intercalated nucleus

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The vomeronasal organ is important for detection of conspecific and heterospecific chemosensory signals and relays information to the medial amygdala via the accessory olfactory bulb. Anterior and posterior regions of the medial amygdala (MeA, MeP) project to preoptic and hypothalamic areas involved in production of appropriate social behaviors. Responses in the medial amygdala may represent an evaluation of chemosensory signals, categorizing stimuli based on salience. In hamsters, immediate early gene FRAs expression increases in MeP as well as MeA after exposure to conspecific chemosensory stimuli, while exposure to heterospecific stimuli increases FRAs expression only in MeP. The medial-caudal intercalated nucleus (m-ICNc), a group of mostly GABAergic neurons lateral to MeP, may modulate MeP activation, preventing a response to non-relevant stimuli. After exposure to heterospecific stimuli, FRAs expression in the hamster m-ICNc is increased while MeP FRAs expression is suppressed and the inverse is true for responses to some conspecific stimuli. Preliminary slice electrophysiology results suggest a functional connection between m-ICNc and MeP and demonstrate that stimulation of m-ICNc leads to inhibition of MeP neurons. Intercalated cell clusters (ITC) elsewhere in the amygdala have also been implicated in the modulation of activity in basolateral and central amygdala of the fear conditioning circuit, via activation of dopamine D1 receptors located on GABAergic ITC neurons. Based on these findings and the immediate early gene expression data it is possible that m-ICNc is involved in shaping MeP response via dopamine D1 receptors on m-ICNc neurons. Ongoing slice electrophysiology experiments with bath-applied drugs, including dopamine agonists and antagonists explore this hypothesis.

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Poster session I Poster #97

Identification of new semiochemicals for *Spodoptera littoralis* from host and non-host plants using GC-SSR and GC-MS

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Spodoptera littoralis(Boisduval) (Lepidoptera: Noctuidae) is a polyphagous pest species on cotton (*Gossypium hirsutum*) and many other agricultural crops and vegetables. Plant volatiles are the predominant cues that drive the host-seeking behaviour of *S. littoralis* female to select a host for feeding and/or oviposition. In a previous study, we used plant odors that are behaviorally and/or electro-physiologically active for *S. littoralis* and other moth species to show that antennal olfactory sensilla of female *S. littoralis* are innervated by different functional classes of olfactory sensory neurons (OSNs). We also found that host leaves have some additional active ligands for OSNs that we did not have in our odor panel tested. In the present study, we have, for the first time, conducted gas chromatography-coupled single sensillum recordings (GC–SSR) on antennal olfactory sensilla of female *S. littoralis* in order to screen for additional putative host attractants and non-host inhibitors. We used air-borne collections from undamaged and conspecific larvae damaged-cotton plants, as well as from flowers of lilac, *Syringa vulgaris*. GC–SSR recordings have so far revealed that a number of OSN types strongly respond to many of the compounds in the biological extracts. The compounds have been subsequently identified through gas chromatography coupled-mass spectrometry (GC–MS). Identification of more bio-active compounds from non-host plants, *Adhatoda vesica* and *Picea abies* that we have shown to inhibit female calling, mating and egg- laying, are in progress.

Poster session I Poster #5

Impact of domestication on the olfactory system of the silk moth *Bombyx mori*

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The domestication of the silk moth *Bombyx mori* started five thousand years ago with the purpose to produce high amounts of silk. *B. mori* is now completely dependent on human care and not able to survive in nature. Because of their nocturnal lifestyle, female silk moths originally had to locate host plants for oviposition mainly by olfactory cues.

We asked which impact domestication had on the olfactory system of *B. mori* by comparing it with that of its wild ancestor *B. mandarina*. We studied anatomical as well as functional modifications using brain reconstructions, electroantennogram recordings, and optical imaging, and found striking differences in *B. mori* as compared to *B. mandarina*. For example, the volume of the antennal lobe, the first olfactory neuropil, was smaller, odour-evoked responses on the antenna were weaker, temporal characteristics of the odour response were modified, and odour-evoked neural activity patterns in the antennal lobe were more variable between individuals. Experiments with hybrids between *B. mori* and *B. mandarina* revealed that some of the observed differences might be linked to the female sex chromosome. We conclude that in the course of domestication the olfactory system of the female silk moth underwent essential changes, reflecting the absent selection pressure on detection and discrimination of plant-derived volatiles. Although *B. mori* has become a model insect for investigations of pheromone detection and processing in males, the olfactory coding of environmental odours seems to be degraded, at least in female *B. mori*. This fact should therefore be taken into consideration when conclusions regarding olfactory physiology and morphology in this species are drawn.

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Poster session II Poster 314

Evaluation of local brain volume alteration in olfactory disorders using voxel-based morphometry

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Several olfactory disorders are known to be associated with structural brain alterations. Especially the olfactory bulb (OB) has been intensively studied using magnetic resonance imaging (MRI). With manual segmentation procedures a volume loss in the OB was documented for quantitative olfactory disorders (e.g. hyposmia) or qualitative olfactory disorders (e.g. parosmia) irrespective of its etiology. Objective of our investigations was the evaluation of volume alterations in higher-order olfactory areas using voxel-based morphometry on 3 Tesla MRI datasets. A quantitative and a qualitative olfactory should be investigated. Hypothesis was a common volume loss in olfactory areas beyond the OB. For this purpose, 24 hyposmic subjects, 22 parosmic subjects and corresponding control subjects matched for age- and sex were included in our study. Voxel-based morphometry (VBM) was performed using the VBM8 toolbox and SPM8 in a Matlab environment. The whole brain analysis revealed significant gray matter volume decreases for hyposmic subjects e.g. in the insular cortex, anterior cingulate cortex, orbitofrontal cortex and piriform cortex. For parosmic patients a volume loss in the left anterior insula was observed. In an additional volume of interest analysis for parosmic subjects including primary and secondary olfactory areas, we also found volume loss in the right anterior insula, the anterior cingulate cortex, the hippocampus bilaterally and the left medial orbitofrontal cortex. Both studied olfactory diseases were reflected by a volume loss in olfactory areas – especially an overlap in the left anterior insular cortex was seen. In summary, quantitative as well as the qualitative olfactory diseases show volume decreases not only in the OB but also in further areas of the central olfactory system.

Poster session II Poster #6

The role of olfaction in gall midge speciation

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Gall midges (Diptera: Cecidomyiidae) are good models to study the role of olfaction in speciation: there are many species compared to other dipterans, they are short lived (1-2 days), driven by olfactory cues and their host plant specificity is a key characteristic. With the swede midge (*Contarinia nasturtii*) as a model, we show that gall midge host specificity is initiated by the olfactory-based host plant choice of the female. We also demonstrate plasticity in the host plant choice, plasticity that may form the basis for fast speciation in the gall midge family. However, host plant volatiles are not the only olfactory cues that affect gall midge speciation. We compare gall midge pheromones with gall midge phylogeny and discuss the role of pheromones in speciation.

Poster session II Poster #396

Neuronal coding of valence of food-related stimuli: intrinsic taste properties versus individual preference

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Previous research has shown that neuronal coding of valence for food-related stimuli can be found in the orbitofrontal cortex, amygdala and anterior insula/frontal operculum (e.g. O'Doherty 2001; Small 2003; Jabbi 2008). However, those studies have used 'universally' pleasant (e.g. sucrose/sweet) or unpleasant (e.g. quinine/bitter) stimuli, providing limited information on whether this coding is due to the intrinsic product properties, or determined by one's individual preferences and experience. In the current study, we use a 'target' food stimulus (grapefruit juice, containing both bitter and sweet components) that is either liked or disliked by subjects, to disentangle intrinsic product properties from individual preferences (extrinsic) and to determine neuronal differences between subjects associated with liking or disliking of a food product.

Forty-eight healthy subjects (24 'likers'/24 'dislikers') will be recruited. In addition to the target stimulus, stimuli perceived as pleasant (sucrose: 70 gr/l) and aversive (quinine: 0.1622 gr/l) by all subjects will be administered, as well as a 'neutral' stimulus (water). Whole-brain BOLD responses will be collected using a 3T Siemens scanner, while tasting the different food stimuli and rating either perceived pleasantness, intensity, familiarity or desirability. Betweengroup (likers vs. dislikers), and between-stimuli (positive, negative, neutral) differences will be analyzed in SPM8. We hypothesize that stimuli with positive valence activate different networks in the brain than negative valence-stimuli, and more specifically that these differences in brain response are mainly seen in the orbitofrontal cortex, striatum, and anterior insula/frontal operculum, brain areas that are involved in food reward and hedonic evaluation. Data collection is currently ongoing; results will be presented at the meeting.

This work was funded by TI food and Nutrition, project number SL-001.

Poster session II Poster #318

Selective ablation of the ENaCα gene in taste tissues alters salt taste perception

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To examine the role of the epithelial sodium channel (ENaC) in salt taste, we generated conditional knockout (KO) mice using the inducible Cre-loxP system. To nullify the expression of the ENaCα subunit (encoded by the *Scnn1a* gene) in taste tissues in a temporally controlled manner, we have produced mice that carry both *Scnn1a*^{flox} and UBC-cre-ESR1 alleles and treated their lingual epithelium topically with an inducer tamoxifen. The tamoxifen treatment resulted in deletion of exon 1 of the *Scnn1a* gene (confirmed by PCR analysis) and nearly complete suppression of *Scnn1a* expression in the lingual tissues but not in the kidneys, stomach, gastrointestinal tract , or lungs (confirmed by quantitative real-time PCR). Immunohistochemistry showed dramatic reduction of the ENaCα protein in the taste tissues of ENaC KO mice, but they did not differ from control mice in taste bud morphology or the expression of several taste-related proteins: phospholipase C β2 (PLCβ2), transient receptor potential cation channel, subfamily M, member 5 (TRPM5), neural cell adhesion molecule (NCAM) or synaptosomal-associated protein, 25kDa (SNAP25). Therefore, *Scnn1a* ablation did not have any generalized effects on taste buds. Taste perception of ENaC KO mice was examined in behavioral studies using two-bottle preference tests, brief-access tests, measurements of NaCl taste thresholds, and analyses of conditioned taste aversion generalization. These studies have shown that the ENaC-dependent salt taste pathway contributes to aversive responses to NaCl, is more sensitive compared with the residual ENaC-independent pathway, and is involved in detection of sodium-specific taste. The system that we have developed for spatially restricted and temporally controlled inhibition of ENaC could also be used to examine function of other genes expressed in lingual tissues.

Poster session I Poster #149

Loss of COUP-TFI function in the Emx1 lineage impairs dopaminergic differentiation in the olfactory bulb

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In the olfactory bulb (OB) GABAergic interneurons are a heterogeneous population deriving from cohorts of spatially segregated progenitors characterized by the expression of defined sets of genes. In this study we show that the orphan nuclear receptor COUP-TFI is expressed in the OB, where it localizes in interneurons both in the granule and glomerular layers. Double labelling for interneuron subtype-specific markers indicates that COUP-TFI largely co-localizes with tyrosine hydroxylase (TH) in dopaminergic neurons. Using genetic ablation of COUP-TFI in Dlx5/6 and Emx1 lineages, we provide evidence that COUP-TFI acts selectively in Emx1-derived progenitors to contribute to the TH-positive olfactory interneuron population. Indeed, loss of COUP-TFI function in the Emx1-, but not in the Dlx5/6-derived lineage, leads to a net decrease in TH-expressing cells. This reduction is not counterbalanced by an increase of other periglomerular cell subtypes and is not attributable to reduced generation or selective loss of dopaminergic-committed cells, as seen by maintenance of the transcription factor Pax6 in these cells, supporting a role for COUP-TFI as an upstream factor critical for TH expression. Moreover, we also show that odor deprivation induces a concomitant reduction in TH- and COUP-TFI-positive cells in the OB, suggesting that COUP-TFI regulates TH expression through an activity-dependent mechanism.

Contributed talks VI "Interactions" Monday 25 June

Identifying a new generation of insect attractants and repellents using intelligent screening of a vast chemical space in silico

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The natural volatile chemical environment consists of a vast array of highly diverse chemical structures that are likely to present thousands of different odors that can be detected by the nose. Most organisms have evolved to depend on successful interpretation of these sensory cues for critical behaviors such as finding mates, avoiding predators, finding food sources, and identifying the best oviposition sites. As a result, these sensory cues can have profound and direct effects on behavior. We have designed a novel chemical informatics pipeline to aid in the identification of physiologically and behaviorally relevant odors. We apply our approach to screen a large collection of 0.5 million odors for ligands of several receptors from a number of different species such as *Drosophila*, mosquitoes, mouse and humans. In rare instances where the receptors for an extremely behaviorally important odor have not been identified, we have devised a method to find improved substitutes based on structural similarities to the known behavior modifiers. We have used a variety of approaches to validate predictions such as single-sensillum electrophysiology and behavior. We show that novel odors identified in this manner can have profound effects on neuronal responses and behavior, demonstrating the effectiveness computational methods can have. Not only do we identify powerful and safe behavior modifying compounds, several new principles of odor coding also emerge from our studies, both at the level of receptor-ligand interactions as well as from the perspective of coding of odors at a systems level.

Symposium 1 "The other noses – the vomeronasal organ, the septal organ and the Grüneberg ganglion" Saturday 23 June

Functional Analysis of Trace Amine-Associated Receptors in Mice

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Olfaction is mediated by a large family of over 1000 canonical odorant receptors in mice. The Trace Amine-Associated Receptors (TAARs) represent a small set of additional chemosensory receptors that are expressed in the main olfactory epithelium. The function of these evolutionarily conserved receptors remains obscure. We have used a combination of gene-targeting to map the inputs of TAAR-expressing sensory neurons to the olfactory bulb, and electrophysiology, *in vivo* imaging, and behavior to investigate TAAR function. We find that a majority of TAARs are mapped to a circumscribed subset of glomeruli in the dorsal olfactory bulb. The sensory neurons and glomeruli associated with this domain are highly sensitive to, and selective for, specific amines. Our data further indicate that the TAAR projection represents a functionally distinct, parallel input pathway dedicated to the detection of a set of volatile odorants that may have specific behavioral relevance. Genetic deletion of the TAAR gene cluster should reveal the contribution of these receptors to olfactory driven behaviors.

Poster session II Poster #136

Selective optical activation of a genetically identified olfactory glomerulus and associated juxtaglomerular neurons

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Olfactory bulb glomeruli are responsible for organizing and relaying sensory information that arrives in the brain. Odors typically evoke activity across multiple glomeruli, which makes it difficult to study how interactions within and between glomeruli shape incoming olfactory information. To overcome this difficulty we used transgenic mice in which channelrhodopsin-2 is selectively expressed in genetically identified olfactory sensory neurons that project to a defined glomerulus. Using these mice, we could specifically activate a single glomerulus via pulsed illumination of the olfactory epithelium with a 447nm laser. To study the activity of neurons associated with this glomerulus, we bulk loaded calcium indicators into the glomerular surround and performed *in vivo* 2-photon optical imaging. Based on this approach, we determined that laser pulses reliably activated approximately 150 juxtaglomerular neurons near the photo-activated glomerulus. Among these neurons we found cells that responded with immediate and fast increases in intracellular calcium (activated cells), while others displayed slower activations. In addition, we detected cells that responded to glomerular activation with decreased intracellular calcium (inhibited cells); unlike activated cells, these inhibited cells appeared to cluster mainly near inactive glomeruli.

Poster session I Poster #137

Interspecific investigations of rodent Grueneberg ganglia.

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In rodents, three well described olfactory subsystems have been reported for odorant and pheromonal discrimination; the main olfactory epithelium, the septal organ of Masera and the vomeronasal organ. Recently, in mice, the Grueneberg ganglion (GG) has been proposed as an additional olfactory subsystem implicated in thermo- and chemo-detection with a specific involvement in alarm pheromone detection. No evidence for this conserved olfactory function has been reported yet in other species. In this study, we used a combination of histological and physiological techniques to investigate the potential presence as well as the function of a GG in different rodent species. We found, by scanning and transmitted electron microscopy, the presence of a GG in rats, hamsters and gerbils. The gross anatomy of these GGs strongly depends on the general morphology of the respective noses. Groups of, or isolated, large cells of different shapes are observed depending on the species. We then performed a comparative morphological study focusing on the two different cell populations present in the GGs and then on the conserved olfactory features of the large cells. As we previously described in mice GG, fine ciliary processes organized in clusters and deeply invaginated in the neuronal soma were observed. These primary cilia are mostly trapped in ensheating glial cells. Using immunohistochemistry, we were also able to confirm the conserved expression and localization of key olfactory transduction elements, such as the membrane ganylyl cyclase G and the cyclic nucleotide-gated channels 3. With a combination of physiological approaches such as calcium imaging and behavioral experiments, we are now performing investigations on the potentially conserved olfactory modalities of these rodent GGs. The presence of a functional Grueneberg ganglion through rodentia species confirmed the essential role of this olfactory subsystem for the survival of the animal.

Poster session I Poster #7

Parallel odor processing in the honeybee favors synaptic coincidence coding

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Honeybees possess an elaborated olfactory system and a rich diversity of odor guided behaviors. Information from olfactory receptor neurons (ORNs) on the antennae is transferred to the glomeruli in the antennal lobe (AL). Two separate uniglomerular projection neuron (PN) output-tracts, the medial and the lateral antennal lobe protocerebral tracts (m- and l-APT), project to higher-order centers in the mushroom bodies (MB) and lateral horn. This dual olfactory pathway is a unique feature in Hymenoptera (Rössler and Zube 2011, ASD 40:349; review: Galizia and Rössler, 2010 Ann Rev Entomol 55:399).

Using multiple wire electrodes (adapted from Strube-Bloss et al. 2011, J Neurosci 31:3129) and a custom-built multi-unit recording setup, we simultaneously recorded and analyzed responses of m- and l-APT PNs. To trace the electrode recording sites, we developed a double-labeling technique to 3D-reconstruct neuronal tracts and electrode positions. Simultaneous recordings from multiple units of both tracts revealed that m- and l-APT PNs respond to a similar set of general odors, pheromones and behaviorally relevant odors. L-APT responses were faster and stronger indicating strong PN recruitment and less accurate odor-quality coding. Responses from m-APT units had longer latencies, showed weaker responses but more complex temporal firing patterns indicating weak PN recruitment, but high accuracy for identity coding. This suggests that the dual olfactory pathway supports parallel odor processing and, potentially, coincidence coding at the level of MB intrinsic neurons (Kenyon cells, KCs). This is supported by odor specific differences in temporal overlap and latency differences between both tracts as calculated with the n-shift algorithm (Nawrot et al. 2003, Biol Cybern 88,321). Parallel processing and coincidence coding of different features from similar odors via two APTs likely enhance the odor coding capacities in this highly olfactory insect.

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Symposium 1 "The other noses – the vomeronasal organ, the septal organ and the Grüneberg ganglion" Saturday 23 June

The other nose : the Grueneberg ganglion

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In mammals, the most recently discovered olfactory subsystem, the so-called Grueneberg ganglion (GG), is present at the tip of the nose, close to the opening of the naris. This organ was first described by Hans Grueneberg in 1973, as a 'ganglion' of unknown function. With the histological methods available at the time, Grueneberg concluded that this ganglion-like cell mass might belong to the complex of the Nervus terminalis. These cells were "rediscovered" in 2005 thanks to the inspection of whole-mount specimens from one particular gene-targeted mouse strain called OMP-GFP. These mice express GFP as a histological reporter under the control of the OMP promoter. OMP (olfactory marker protein) is a mature olfactory-sensory neuron-specific marker. In memory of the original work of Grueneberg, this structure kept its original name. The GG is an arrow-shaped neuronal structure at the anterior end of the nasal cavity that lines both sides of the nasal septum. The paired organ is located in a shallow depression of the cartilage. In an adult mouse, from 300 to 500 cells can be found in each GG. It has been recently shown by a variety of technical approaches that the GG is implicated in thermo- and chemo-detection with a specific involvement in alarm pheromone (AP) sensing. A GG comprises two populations of cells : ensheating glial cells and neurons bearing multiple primary cilia, putative site of sensory transductions. Membrane proteins that might be important for the specific chemosensory function taking place in this ganglion from birth on: danger detection via AP recognition have now been identified. AP evoked calcium responses in GG neurons in vitro and induced freezing behavior in vivo, which completely disappeared when the GG degenerated after axotomy. Chemosensing by the GG is a typical example of how the nervous system has adapted to sense and react to a very important social cue. AP sensing is an instinctive or innate behaviour involved in natural selection.

Poster session II Poster #8

Genome-wide association mapping of natural variation in odor-guided behavior in *Drosophila*

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Genome-wide association (GWA) studies are a powerful tool for providing fine scale resolution of the genetic basis of behavioral variation. With the recent sequencing of a panel of *Drosophila* inbred lines derived from a natural population we can now take advantage of this approach to understand the genetic architecture of behavior. Here, we conduct GWA studies of odor-guided behavior in *Drosophila melanogaster*. Olfactory signals are used by many animals to gain information about their environment, such as the identification of appropriate feeding and breeding substrates. We have designed a high-throughput behavioral assay system that allows for the assessment of both temporal and spatial dynamics of odor-guided behavior. We observed significant variation among wild-derived lines in several odor-guided behavior phenotypes in response to 2,3-butanedione, a volatile compound present in fermenting fruit. Our GWA analyses revealed numerous single nucleotide polymorphisms (SNPs) associated with variation in odor-guided behavioral responses, with partially non-overlapping sets of polymorphisms contributing to the spatial and temporal dynamics of odor-guided behavior. Of the GWA candidate genes, both novel and previously identified olfactory-related genes were found. Global gene network analyses revealed that genes influencing variation in odor-guided behavior are enriched for functions involving neural processing and that these genes form a pleiotropic network of interactions. In short, our results showed that subtle changes influencing nervous system development can result in profound differences in variation in behavior.

Poster session I Poster #321

Taste interactions among sucrose, sucralose and ethanol in hamsters

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Sucralose (a chlorinated sugar analog), ethanol (EtOH) and sucrose share taste similarities in hamsters (*Mesocricetus auratus*), judged by generalization of conditioned taste aversions (CTA) and chorda tympani (CT) nerve activation. Unlike humans, hamsters do not drink alcohol for its intoxicating effects because they metabolize alcohol rapidly. EtOH CTA generalize to sucrose and sucrose CTA generalize to sucralose. All 3 compounds, which differ in caloric value (EtOH > sucrose > sucralose), may activate T1R receptors but EtOH and sucralose may also activate T2R receptors. By pairing LiCl injection with intake in 24 hamsters, CTA were established to 100mM sucrose, 10% EtOH or 1mM quinine-HCl, all of which generalized to 3mM and 10mM sucralose (average 39% suppression, $p < .05$). Aversions to quinine, EtOH, and sucrose were specific to the conditioned stimuli (average 54% suppression). Like human taste, relative hamster CT nerve (n = 6-7) stimulus effectiveness, measured as initial 10-s area-under-curve, was sucralose > sucrose > EtOH at the concentrations used or on a molar basis. Hamster CT responses (0.5M NH_4Cl standard = 100) were 19±4 for 10% EtOH, 32±4 for 100mM sucrose, 48±6 for 3mM sucralose and 51±5 for 10 mM sucralose. This order may relate to ability to bind taste receptors, suggesting receptor processes are similar for humans and hamsters. EtOH and sucrose mixed at low concentrations produced synergistic CT responses ($p = .005$). A possible explanation is activation of T1R2 and T1R3 subunits of the sweet receptor in a combinatorial way combined with the ~50 response saturation. As CT response magnitude increased, synergy gave way to additivity and then to saturation, achieved by 3mM and 10 mM sucralose responses that equaled the mixture response (48±4). Behavioral and neural similarity between sucrose and sucralose indicates they have similar tastes not explained by post-ingestive effects, since sucralose has no caloric value.
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Delwart Contributed Symposium - Higher olfactory processing Tuesday 26 June

Mouse olfactory peduncle: core structure

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The olfactory bulb processes odor information detected in the nasal cavity. The area just behind the olfactory bulb is known as the olfactory peduncle. It contains the rostral-most portion of the olfactory cortex (a region often referred to as the anterior olfactory nucleus) as well as two smaller areas. The peduncle is traversed by two large tracts. The first is the lateral olfactory tract (LOT), composed primarily of axons extending posteriorly from the bulb to the cortex. The second, found in the core of the peduncle, is the "anterior limb of the anterior commissure". This complex tract contains axons coursing anteriorly from a variety of sources, including processes from the contralateral olfactory cortex (that cross in the anterior commissure), fibers from more posterior portions of the ipsilateral olfactory cortex, and diverse fibers entering the tract from the medial forebrain bundle and other areas (e.g., noradrenergic, cholinergic, histaminergic, orexinergic and serotonergic axons). The purpose of the present study was to characterize this deep white matter region and to compare it with an earlier examination of the LOT (Brunjes et al., *J. Comp. N*eurol. 519, 2011). Reconstructions of the tract from an anterior and a posterior region of the peduncle were produced at 800X via electron microscopy. Every myelinated axon in the two areas was outlined, yielding a total data set of almost 87,000 profiles. This population was then sampled to visualize the distribution of the largest and smallest caliber axons and profiles with an elongated shape (and thus travelling obliquely to the plane of section). While local areas exhibited differences in each of these categories, no large scale patterns of organization were observed. Mean axon caliber was significantly higher in the anterior region of the peduncular core, and profiles seen in both regions were smaller than those observed in the LOT. Supported by Grant DC000338 from NIH (NIDCD).

Poster session II Poster #298

Olfactory performance of patients suffering from autism spectrum disorders (ASD)

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Autism spectrum disorders (ASD) are clinically characterized by a triad of symptom clusters, namely challenges in social interaction and communication as well as repetitive behaviors. Furthermore, ASD represents an innate and neurodevelopmental psychiatric disorder with dysfunctional sensory perception and neural processing. Changes in chemosensory function have been identified in several neurodegenerative or psychiatric disorders, like Parkinson's and Alzheimer's disease or depression. Knowledge about chemosensory function in adult ASD patients is sparse due to a small number of patients included in studies and a limited number of different olfactory subtests that have been carried out. Taken together, it is difficult to draw comprehensive conclusions from the existing studies. Therefore, our aim was to compare olfactory performance of 30 adult ASD patients with 30 age- and gender-matched healthy control subjects. Two independent clinicians made the diagnosis. Furthermore, patients underwent a concise battery of psychological tests including the Autism Diagnostic Observation Schedule (ADOS). We obtained olfactory performance scores by using the Sniffin' Sticks threshold test (n-butanol), odor discrimination test, and identification test (MONEX-40) as well as pleasantness and intensity ratings. Our results suggest that olfactory sensitivity and olfactory pleasantness evaluation is diminished in patients suffering from ASD compared to healthy control subjects. Even after exclusion of patients that were smoking, taking medication, or suffering from comorbidities and their corresponding control subjects these results are stable. It is suggested that the decline in olfactory sensitivity and pleasantness ratings are distinguishing characteristics of ASD patients in comparison to healthy controls. The potential of using olfactory performance scores as future biomarkers will be discussed.

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Delwart Contributed Symposium - Higher olfactory processing Tuesday 26 June

Carbon dioxide avoidance requires context dependent mushroom body activity in *Drosophila*

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Animals and humans react to odours in their environment with aversion or attraction. While aversion and attraction are largely innate, inner state as well as environmental conditions greatly influence an animal's choice between these two reactions. Frequently, a conflict between two opposing stimuli arises, forcing the animal to take a context-dependent decision. The neuronal basis for these behaviours is studied extensively in *Drosophila melanogaster*. Here, we show that the generally aversive odour CO_2 can be overcome, when a fly is hungry. Using *in vivo* neuronal silencing to dissect the underlying circuitry, we show that the mushroom body (MB), a centre for learning and memory, is essential for innate CO_2 avoidance behaviour exclusively in the context of starvation. Further more, we identify a novel CO_2 projection neuron that targets the MB directly. These findings show that a learning and memory centre is also essential for context and inner state dependent innate behaviour.

Plenary lecture Tuesday 26 June

Olfactory mechanisms in mammals – Delwart Lecture

Linda Buck

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The sense of smell allows mammals to perceive myriad chemicals as having a distinct odor. It also mediates the detection of pheromones that elicit innate responses. How does the olfactory system detect so many different chemicals and how does the nervous system translate those chemicals into diverse perceptions and behaviors? Using a combination of molecular, cellular, and genetic approaches, we have identified families of receptors that initially detect odorants and pheromones in peripheral sense organs, asked how those receptors encode the identities of different chemicals, and investigated how the signals they generate are routed and organized in the nervous system to yield distinct perceptions and instinctive responses.

Poster session II Poster #424

Volatile, odorous substances travelling through the human body – uptake, distribution, removal … function?

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Volatile and odorous substances are candidates that are likely to enter the human body by different routes, pass through diverse physiological compartments, at times with quite few limitations, and may elicit various physiological actions by potentially fitting into diverse size-restricted physiological target sites; these manifold features of odorants relate to their relatively small molecular size and their high mobility in gaseous, liquid, and even solid phases. Science from different disciplines is just about to explore this fascinating field of odorant / volatile interaction *in vivo*, with processes that go far beyond the conventional perception of smell.

With regard to the exploration of such physiological phenomena, the underlying (bio-) chemical transformations and transitions of volatiles /odorants within the human body are similarly attracting increasing attention.

Investigations focus, as far as possible, on monitoring the actual molecules involved *in vivo* or, at least, in physiological systems as close as possible to the respective physiological conditions. To deal with the challenging issues of selectivity, sensitivity and temporal resolution, novel techniques are developed and adapted for the specific analytical requirements. In the course of such studies, it becomes increasingly clear that the human physiology not only senses and reacts to signals obtained via volatiles / odorants, but also exerts quite pronounced (bio-) chemical modulations on such substances that in turn result in new compounds that may further exert other potential physiological effects.

An overview of state-of-the-art analytics applied to monitor such substances in *in vivo* processes, together with discussion of the respective (bio-) chemical principles will be provided in the presentation.

Poster session II Poster 190

Agonist profiling of the mouse formyl peptide receptors reveal a stereoselective tuning of mFpr-rs1

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Recently several members of the mouse formyl peptide receptor (Fpr) family were discovered in the vomeronasal organ (VNO), the key detector of pheromones and similar semiochemicals. The biological role of these Fpr receptors is yet not clear. However, their homology to the human FPR-family that contributes to host immune defense suggests that Fpr-receptors in the VNO may be involved in pathogen detection. A precise knowledge about their ligand profile provides helpful insights towards unraveling their function. We tested all seven mouse Fpr-receptors in heterologous systems by functional high-throughput calcium imaging with 29 compounds covering many prototypical ligand classes. We observed broad tuning and an intriguing functional overlap between human FPRs, mFpr1 and mFpr2. 21 of the 29 compounds activated either mFpr1 or mFpr2 that are expressed in mouse leucocytes, and most likely fulfill similar functions as their human counterparts. In marked contrast Fpr-related receptors (Fpr-rs) expressed in the VNO are much more narrowly tuned. Surprisingly, we observed that mFpr-rs1 is stereoselectively activated by a family of peptides containing D-amino acids. These pharmacologic properties of mFpr-rs1 are consistent with its proposed role in pathogen detection because similar peptides are known to exist in bacteria and fungi. To test the biological significance of these findings we challenged acutely dissociated VNO neurons from OMP-GFP animals with a range of selected ligands in a novel high-throughput calcium imaging assay. We observed several pharmacologically distinct subpopulations. Intriguingly, 2% of the cells showed a stereoselective agonist profile that is similar to that of mFpr-rs1 in the heterologous system. Currently, we are investigating the underlying molecular signal transduction and behavioral response to these compounds. Supported by DFG-grants SFB894, INST 256/273-1 FUGG and Volkswagen Foundation.

Poster session II Poster #398

Pulsation induced taste enhancement: why continuous alternation makes sense

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The successive alternation of high and low tastant concentrations (pulsatile stimulation) results in higher taste intensity than stimulation with the same net but non-alternating tastant concentration (continuous stimulation) [1-3]. We conducted several studies to elucidate the underlying mechanism of pulsation induced taste enhancement. We investigated the effects of pulsation rate [3-4], pulse-interval contrast [5], taste quality and taste transduction mechanism [6] on the taste intensity enhancement. In this contribution we summarize the key findings of those studies and propose a mechanism to explain our findings. This mechanism is based on enhancement effects at preconscious stages of gustatory processing.

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Poster session II Poster #138

Antiobesity and antihyperglycemic effects of cinnamaldehyde in mice.

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Beyond the taste function of spices, numerous health benefits have been ascribed to naturally derived flavoring molecules. However, the molecular mechanisms by which these molecules mediate their effects remain largely unknown. Cinnamaldehyde (CIN) is an organic compound that gives cinnamon its flavor and odor.

In the present study, the physiological effects of CIN after ingestion were studied in a mouse model. To explore the long-term effect of CIN administration, a protocol for daily ingestion of CIN at 0.2% in high fat diet for 5 weeks using diet induced obese C57BL/6 mice was developed.

No effect on food intake was observed but body weight gain decreased significantly (13.4%) and this effect has been observed before (Huang et al., 2011). Fasting blood glucose levels were significantly lower in mice treated with CIN. Moreover, mice showed significantly decreased glucose response to an oral glucose tolerance test (OGTT). Plasma insulin levels in the fasting state and during the OGTT were unchanged by the CIN treatment. In addition, the mice showed no differences in plasma triglycerides, free fatty acids and cholesterol levels. Finally, using NMR, a reduction in fat mass gain was also observed.

Improved glucose tolerance without a change in the plasma insulin concentration suggests enhanced insulin response to glucose ingestion. This improvement in oral glucose tolerance with CIN could result from either enhanced peripheral tissue (muscle) sensitivity to insulin or an improvement in hepatic insulin sensitivity. Reduction of fat mass suggests also an increase of cellular metabolism.

In conclusion, dietary CIN administration could be used as a potential antiobesity and antihyperglycemic strategy.

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 253541.

Poster session I Poster #293

The effect of repeated testing, sex, and the menstrual cycle on electrogustometric thresholds.

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Introduction: Enhancement in discriminative responses following exposure to specific stimuli has been investigated in most sensory systems. The effect of prior exposure and potential perceptual learning on taste thresholds, particularly as measured by electrogustometry, appears less well studied. The current study was designed primarily to examine the effect of repeated testing on electrogustometic thresholds. A second goal was to explore sex differences in such thresholds and a third goal was to explore whether these thresholds are affected by the menstrual cycle.

Method: Thresholds were studied longitudinally. Testing occurred every other day for 1 month in 6 young adults (3 males, 3 females) aged 19 to 21 years (Exp 1), every day for 5 weeks in 13 young adult females (Exp 2) and three times over the course of one menstrual cycle in 34 females of childbearing age (Exp 3). Taste thresholds were measured at 2 locations at the back of the tongue (Exp. 2) or 4 locations at the front and back of the tongue (Exps 1 and 3) corresponding to the left and right chorda tympani and glossopharyngeal nerves using a standard 1-up, 2-down staircase method. Phase of the menstrual cycle was determined by basal body temperature and One Step LH Urine Test, which indicates a surge in luteinizing hormone.

Results: Thresholds decreased across test sessions in all three experiments (a trend in Exp. 3). Thresholds were lower in females than males at all tongue locations (Exp. 1). Menstrual cycle data (from all three experiments) suggest a decrease in threshold at mid-cycle, but are not conclusive.

Conclusion: Overall females exhibited higher taste sensitivity. Both sexes demonstrated enhancement in discriminative responses across test sessions. The effect of the menstrual cycle is suggestive, and further research is needed to explore the influence of hormones on taste sensitivity.

Poster session I Poster #89

Functional imaging of population coding in olfaction: neural activity to perception

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A central goal in neuroscience is to understand the relationship between neural activity and sensory perception. How different do two response patterns have to be in order to be perceived as distinct? This difference can be considered a unit of the neural code - the smallest difference in activity that the animal can perceive. We have addressed this question in the olfactory system of Drosophila, focusing on a brain area known as the Mushroom Body (MB), which is essential for learned olfactory discrimination. Using imaging techniques to track over half the population of MB neurons, we address this question with a completeness not currently feasible in mammalian systems.

We compared behavioral measures of olfactory discrimination with neural activity patterns monitored using calcium imaging. Flies were trained to form a Pavlovian association with one odor, and then chose between the trained odor and a different odor in a T-maze. To track neural activity, we targeted expression of the calcium sensor, GCaMP 3.0, specifically to MB neurons using genetic tools available in Drosophila. We used two-photon imaging to record activity patterns, incorporating a piezoelectric z-motor to image the entire MB in a 3-dimensional volume during a single odor presentation. This approach enabled us to routinely monitor neural activity of ~75% of the total population of MB neurons. By measuring odor responses on a trial-by-trial basis, we could accurately quantify response variability. This enabled us to construct an algorithm to classify odors based on the patterns of active neurons, taking into account the noise inherent in neuronal responses.

MB odor responses were sparse, consistent with electrophysiological recordings. Different monomolecular odors could be readily distinguished from one another based on activity patterns, even when odors evoked exceptionally similar

patterns of activity in the population of Olfactory Receptor Neurons. Behavioral tests showed a similar level of discriminability. We then tested a series of progressively similar binary blends of odor, which enabled us to construct a psychometric curve characterizing discriminability as a function of stimulus similarity. We established a corresponding neurometric curve describing our ability to classify odors based on those activity patterns. There was a close correspondence between the neurometric and psychometric curves, indicating our analysis reflects the animal's capacity to discriminate odors. Overall, our results show that small differences in input are nevertheless easily distinguished in the MB population, a transformation that likely underlies accurate memory storage.

Poster session I Poster #125

Olfactory sensitivity and odor structure-activity relationships for aliphatic carboxylic acids in CD-1 mice

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Using a conditioning paradigm, the olfactory sensitivity of CD-1 mice for a homologous series of aliphatic n-carboxylic acids (ethanoic acid to n-octanoic acid) and several of their isomeric forms was investigated. With all 14 odorants, the animals significantly discriminated concentrations as low as 0.03 ppm (parts per million) from the solvent, and with four odorants the best-scoring animals even detected concentrations as low as 3 ppt (parts per trillion). Analysis of odor structure-activity relationships showed that the correlation between olfactory detection thresholds of the mice for the unbranched carboxylic acids and carbon chain length can best be described as a U-shaped function with the lowest threshold values at n-butanoic acid. A significant positive correlation between olfactory detection thresholds and carbon chain length of the carboxylic acids with their branching next to the functional carboxyl group was found. In contrast, no such correlation was found for carboxylic acids with their branching at the distal end of the carbon chain relative to the functional carboxyl group. Finally, a significant correlation was found between olfactory detection thresholds and the position of the branching of the carboxylic acids. Across-species comparisons suggest that mice are more sensitive for short-chained (C_2 to C_4) aliphatic n-carboxylic acids than other mammalian species, but not for longer-chained ones (C_5 to C_8). Further comparisons suggest that odor structure-activity relationships are both substance class- and species-specific.

Poster session I Poster #139

Concentration-dependent variation in sparseness of odor coding in the mouse olfactory bulb

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In mammals, odorant molecules are sensed by a large family of receptors expressed by sensory neurons projecting their axons in a receptor specific manner onto olfactory bulb (OB) glomeruli. Each odorant is thought to activate only a few glomeruli and thereby a limited number of output neurons in the bulb, which has led to the hypothesis that odor coding in the OB is sparse. However, the studies that support this model used anesthetized animals or monomolecular odorants at a limited concentration range. In this study, we evaluated odor coding in awake mice using natural stimuli. Using optical imaging, tetrode recordings and 2-photon microscopy *in vivo*, we show that natural odorants at their native concentration activate a large fraction of the glomeruli and that OB output neurons are more broadly tuned than previously thought. By decreasing the odorant concentration, we observe a sparsening of the activated glomeruli patterns and we report that OB output neurons become more narrowly tuned. We conclude that the sparseness of the odor code can strongly vary with the strength of sensory stimuli.

Delwart Contributed Symposium - Higher olfactory processing Tuesday 26 June

Deconstructing odor representations in the mushroom body

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In the sensory environment of *Drosophila*, most odors do not carry an a priori meaning. Rather, they can elicit appetitive or aversive behaviors depending on the context in which they were first perceived. It is currently unclear how neural circuits are organized to allow for such context-dependent associations. At the peripheral level, the *Drosophila* olfactory circuit is a highly stereotypical map: neurons expressing a given odorant receptor converge into a common glomerulus in the antennal lobe. How the olfactory circuit is organized at the next processing level, the mushroom body, a brain center required for learning and the formation of associative memories, is not known. Here, we characterize the connections between Kenyon cells, the major cell type in the mushroom body, and the projection neurons that innervate individual glomeruli. We combine neural tracing using a photoactivable green fluorescent protein (PA-GFP) with single-cell dye-injection to determine the number and identity of projection neurons connected to individual Kenyon cells. We find that Kenyon cells are connected randomly to the antennal lobe: Kenyon cells sample widely across glomeruli and integrate input from different sensory modalities. This coding strategy is in contrast to the stereotypical map seen at the periphery and is well suited for context-dependent associations as it represents sensory information without an a priori bias.

Poster session I Poster #85

Electrophysiological and behavioural responses of the Grapevine Moth to odours of *Perilla frutescens*

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The Grapevine Moth *Lobesia botrana* is a major pest of grapes worldwide and its control still largely relies on insecticide applications. There is accordingly great interest in identifying attractant or repellent semiochemicals interfering efficiently with both male and female olfaction, in order to develop alternative control strategies.

Host plant volatiles playing a relevant role in the plant selection process of this insect have been extensively studied but their effectiveness for control purposes is strongly negatively affected by the overlapping background odour in the vineyard. Finding of behaviourally active compounds emitted by non-host plants would represent an attractive target for the *L. botrana* control. Therefore, we studied the biological activity on *L. botrana* olfactory system of secondary metabolites isolated from the Asian food plant *Perilla frutescens* (L.). Interestingly, *Perilla* compounds were shown to activate a novel family of receptor, the Transient Receptor Potential (TRP) channels, expressed also in the antennae of lepidopterous species.

The olfactory response of *L. botrana* females to *Perilla* extracts was preliminary tested adopting electroantennographic experiments (EAG). Antennal-active compounds were identified by a gas chromatograph equipped with a mass spectrometer (GC-MS). Moreover, gas-chromatography coupled with electroantennography (GC-EAD) allowed to detect several active compounds eliciting significant electrophysiological responses on female antennae. In a dual choice oviposition test based on olfactory cues, females showed a dose-dependent preference for the odours released by *Perilla* even in presence of the odour bouquet of the host plants.

Future molecular, physiological and behavioural studies will focus on the activity of the single and blended *Perilla* compounds and on the role of TRP receptors in their perception with the aim to improve the current strategies for the management of pest populations.

Poster session I Poster #141

Responses to sulfated steroids of female mouse vomeronasal sensory neurons

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In mammals, chemoreception takes place in two organs which are the major sites of chemical recognition: the main olfactory epithelium and the vomeronasal organ (VNO). The VNO is an accessory olfactory organ located at the base of nasal cavity and plays important role in many behaviors.Vomeronasal sensory neurons (VSNs) are activated with high efficacy by sulfated steroids (products of steroidal hormones catabolism), which are predominant ligands in female-mouse urine. We have measured the intracellular calcium concentration changes induced by the application of sulfated steroids to VSNs isolated from female mice, using the calcium dye fluo-4. We found that a mix of ten sulfated steroids from the androgen, estrogen, pregnanolone, and glucocorticoid families induced a calcium response in 71% of VNs, a higher percentages compared to the frequency of urine response (28%). Moreover, 31% of the VSNs responded to a mix composed of three glucocorticoid-derived compounds, and 27% responded to a mix composed of three pregnanolone-derived compounds. None of the VSNs that responded to one mix responded also to the other, indicating that responses were highly specific. Immunohistochemistry showed that the VSNs responding to sulfated steroids expressed phosphodiesterase 4A, a marker specific for apical VSNs expressing V1R receptors. Some VSNs responded to more than one individual component of the glucocorticoid-derived mix, suggesting that these neurons are broadly tuned; on the contrary, other VSNs responded only to one compound of the pregnanolone-derived mix. However, both types of VSNs still displayed strong specificity, remaining unresponsive to high concentrations of the ineffective compounds

Poster session II Poster #142

Analysis of female reproductive behavior in conditional G(alpha)o-deficient mice

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We have recently created a conditional Gαo-Cre-loxP mutant mouse strain (cGαo–/–) in which the G-protein Gαo has been deleted from sensory neurons of the vomeronasal organ (VNO) (1). Gαo is essential for the transduction of peptide and protein pheromones in the basal half of the VNO and for the display of male–male territorial aggression as well as maternal aggression (1). Here we have begun to analyze the role of Gαo-positive VNO neurons and the consequences of the partial loss on VNO function in female sexual behavior. Previous data on mice deficient for the VNO signal transduction channel Trpc2 indicate that female VNO signaling normally inhibits the activity of neural circuits that control the expression of male typical mating behavior by female mice (2). Our analysis of sexual behavior in cGαo–/– female mice showed no significant increase in male-specific behaviors towards other females, males or castrated mice. We quantified parameters that are indicative of male-typical sexual behavior such as mounting behavior and pelvic thrusting. The number of cGαo–/– females showing mounts towards other mice as well as the number and duration of mounting behavior and pelvic thrusts remained low in all cases or were even absent. Other social interactions such as aggression and sniffing towards the intruder mouse remained unchanged in the cGαo–/– females. Currently, we are investigating whether Gαo-dependent VSN signaling is essential in other aspects of sexual and reproductive behaviors of female mice including facilitation of sexual receptivity (lordosis) and selective pregnancy block (Bruce effect). These experiments should provide new insights into the role of the Gαo-expressing VNO subsystem in the expression of sex-specific behaviors in mice.

This work was supported by the Deutsche Forschungsgemeinschaft, NIH Intramural Research Program and the Volkswagen Foundation.

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Poster session I Poster #147

Pheromonal odour learning and brain activation in the newborn rabbit

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Mother-young relationship strongly depends on olfaction. Maternal odour cues contribute for instance to arousal, attachment, and also directly to the mammae localization and sucking. European rabbit pups (*Oryctolagus cuniculus*) display a typical orocephalic behaviour to locate and grasp the nipples during nursing. This behaviour is triggered, among different cues, by the mammary pheromone (MP) emitted by lactating females. Besides its releasing role, the MP efficiently promotes the learning of new, initially neutral odorants. In this form of associative conditioning, after single and short pairing with the MP, the learned odorant becomes able to trigger the neonatal orocephalic behaviour as efficiently as the MP. We previously used Fos neuroimaging to examine the brain network activated by the MP in 4-day- old pups. It revealed a large activation of the main olfactory bulb (MOB) and a specific labeling of central structures (e.g., OVLT; hypothalamic lateral preoptic area, LPO). Here, we investigated the activation generated by a neutral odorant (ethyl-acetoacetate; EAA) learned by association with the MP or not learned, to highlight the reorganization of the neuronal substrate that follows the conditioning and sustains the behavioural response. After conditioning/pseudo- conditioning to EAA on day 3 and re-exposure to EAA on day 4, the brain of pups was processed by Fos immunocytochemistry. In the MOB, the immunolabeling was higher in conditioned rabbits, and higher in their mitral+granule cells compared to their glomerular layers, whatever the rostro-caudal level. Differences also appeared in regions and sectors of the glomerular layer in both groups. Contrasted activation appeared also in higher brain regions of conditioned pups such as the piriform cortex and the amygdala (i.e. "learning" structures) and in the LPO (implicated in osmoregulation). Thus, MP-induced odour learning is followed by changes in the neonatal brain processing of EAA before/after acquisition.

Poster session II Poster #240

Heritability of enantioselectivity in human olfaction

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To the human nose, optical enantiomers could possess different smells. Here we probe the genetic contribution to such enantioselectivity in the human olfactory system, where phenotypic diversity is widespread. In a four-trial three-alternative forced choice test, we assessed the abilities of monozygotic and dizygotic twins to distinguish between the enantiomers of carvone, limonene, as well as α-pinene, with one, one, and two chiral centers, respectively. We identified a reliable genetic component in the chiral discriminations of carvone and limonene, but not α-pinene enantiomers. Whereas the olfactory coding mechanism underlying the discrimination between enantiomers is scantily known, our findings suggest that different chiral carbons are independently encoded in the nose.

Poster session I Poster #143

Detection of acidic pH in the mouse vomeronasal organ

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The mouse vomeronasal organ (VNO) plays a key role in pheromone detection and recognition of other social signals. However, the underlying mechanisms of signal detection in the VNO remain largely unknown.

Here, we describe activation of vomeronasal sensory neurons by extracellular protons. To investigate the mechanisms involved, we performed whole-cell patch-clamp recordings from visually identified sensory neurons in acute tissue slices of

the mouse VNO. We show that acidic solutions of different pH values elicit robust action potential firing in current-clamp recordings. The same stimuli dose-dependently induce inward currents in voltage-clamp measurements. The ionic characterization of the underlying conductance and the pharmacological profile of the acid-induced responses indicate a possible involvement of different proton-sensitive ion channels and receptors.

On-going biochemical and molecular investigations as well as electrophysiological measurements will provide insight into the functional role of proton-detection in the vomeronasal organ of mice.

Contributed talks I "Modulation of the olfactory system (Linnaeus Symposium)" Monday 25 June

Mechanisms of intrinsic learning within olfactory bulb

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Transformations of odor representations in the olfactory bulb depend substantially on learning. Intrinsic long-term memory within olfactory bulb (based on the selective survival of adult-born neurons) has been clearly established, but the short-term memory mechanisms preceding and defining the induction of these long-term bulbar memories remain unclear. We first investigated the role that muscarinic modulation of bulb circuitry plays in olfactory associative learning. We found that intrabulbar infusion of scopolamine impaired olfactory learning when delivered between training and testing, or when delivered prior to training in studies imposing a similar 45-minute training-testing latency, but not when testing followed training immediately or with a four-minute latency. This pattern of results indicates that intact muscarinic responsivity within OB is important for the maintenance of an intact odor memory over this delay period. Odor memory over longer timescales additionally may depend on brain-derived neurotrophic factor (BDNF) signaling within olfactory bulb. Interestingly, isoflurane anesthesia independently generated retrograde amnesia for learning occurring prior to the onset of anesthesia. Intrabulbar infusion of the fast glutamate reuptake inhibitor dihydrokainate (DHK) rescued this amnestic effect for olfactory learning, but not for a comparable visual learning paradigm. As DHK does not appear to interact with scopolamine, its use facilitated the infusion of scopolamine between training and testing in order to differentiate learning from recall effects. Memory mechanisms within olfactory bulb enable adaptation to natural olfactory scenes and are likely to be crucial for effectively deploying sensory resources in the high-dimensional olfactory modality. Supported by NIDCD grant DC009948.

Poster session I Poster #299

Olfactory Disturbances in Anxiety Disorders

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The olfactory system plays an important role in both animal and human anxiety reactions. However, results on olfactory performance in patients suffering from clinical anxiety disorders are scarce. Therefore, we conducted an exploratory pilot study in 17 patients (9 men, 8 women) currently diagnosed with an anxiety disorder (according to DSM-IV). Patients participated in olfactory and psychological testing and were compared to 17 healthy controls. For olfactory testing, the Sniffin' Sticks Test was employed extended by visual analogue rating scales for the assessment of odour intensity and hedonics. Anxiety symptoms were assessed by means of the Beck Anxiety Inventory. Statistical analyses revealed significant deficits concerning olfactory discrimination in patients, while no differences in threshold and identification ability occurred. Most interestingly, anxiety patients showed significantly higher intensity judgements and an increased rating range concerning olfactory hedonic estimates. From a clinical perspective, the patients' tendency towards more extreme evaluations of olfactory stimuli may be related to heightened arousal and hyperresponsiveness often seen in anxiety disorders. At an anatomical level, results highlight the role of the amygdala, in both human fear circuitry and subjective odour perception.

Poster session I Poster #9

Role of xenobiotic metabolizing enzymes in the perception of caffeine in *Drosophila melanogaster*.

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Xenobiotic metabolizing enzymes (XME), such as cytochromes P450 (CYP), UDP-glycosyltransferase (UGT) and glutathione-transferase (GST) can take in charge exogenous potentially toxic molecules, biotransform and eliminate them out the organism. XME have been detected in chemosensory organs and are thought to modulate the chemoperceptive process by neutralizing the stimulus molecule after its detection, avoiding the saturation of receptors, this allowing the neuron to quickly respond to another stimulus.

The aim of our study consisted to investigate the role of XME in the sensory perception of caffeine in *Drosophila melanogaster*. We performed a transcriptomic study on adult male flies exposed to caffeine in order to identify all XME genes whose expression was modulated by an exposure to this molecule. The mRNA expression of different CYP, UGT and GST genes was up-regulated in chemosensory organs after the caffeine exposure. This result suggests that these XME genes could be involved in the metabolism of caffeine in these tissues.

We hypothesized that a modulation of the CYP candidate should trigger a modification of caffeine detection and alter feeding behaviour. We targeted the RNAi of candidate XME genes to down regulate their expression in different sensory cells. Using the MultiCAFE behavioural test, to measure the consumption of a caffeine solution, we found that the ability of transgenic flies to detect caffeine was altered. Therefore, the decrease of several CYP genes expression in specific subsets of sensory neurons was correlated with altered feeding behaviour.

These data suggest that the expression of some XME genes in sensory organs is required for caffeine perception by insects.

Symposium 20 “Aquatic olfaction” Tuesday 26 June

Identification and localization of olfactory-specific ionotropic glutamate receptors in lobster olfactory receptor neurons

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Until recently, the nature of the olfactory receptor (OR) in crustaceans, a major group of arthropods, has remained elusive. Notwithstanding accumulating evidence that G protein activated metabotropic signaling mediates lobster olfactory signal transduction, we now show that an earlier reported olfactory-specific ionotropic glutamate receptor (OET07; Hollins et al 2003) appears to be an ortholog of the recently discovered *Drosophila* olfactory variants of ionotropic glutamate receptors (IRs). These findings suggest that crustaceans may have IRs, similar to insects, but that are capable of activating metabotropic signaling. Aided by a *Panulirus argus* olfactory transcriptome, we sequenced multiple full length lobster IR orthologs, including OET07 (PaIR1), and demonstrated that these putative IRs can be detected in olfactory tissue by RT-PCR. Unlike the IR expression pattern in insect olfactory receptor neurons (ORNs), most, if not all, lobster ORNs express PaIR1 (ortholog of IR25a), as confirmed by *in situ* hybridization. PaIR1 can be further localized to the transduction compartment (outer dendrites) of ORNs by western blotting and immunocytochemistry. While PaIR1 appears to be a common subunit, other IRs are expressed in very few ORNs, suggesting specific receptor function. Odorant-induced responses from HEK cells heterologously expressing a combination of two PaIRs, 1 and 2, indicate that lobster IRs can form functional ORs and suggests that, as in insects, they function as heteromers. Single biologically-relevant odorants, e.g., amino acids, trigger a calcium response in only 0.3-6% lobster ORNs *in situ*, consistent with the IR expression pattern. The long-standing evidence for odorant-activated metabotropic signaling in lobster ORNs together with the new evidence for expression of IRs in the same cells argues that ionotropic and metabotropic signaling work in concert to effect lobster olfactory transduction. Supported by the NIH NIDCD (DC005995, DC001655) .

Poster session II Poster #144

PI3K-dependent odorant antagonism in mammalian olfactory receptor neurons

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Mammalian odorant receptors (ORs) are well known to be GPCRs coupled to excitatory cyclic nucleotide signaling. Olfactory receptor neurons (ORNs), however, are capable of opponent coding in that they also can be inhibited by odorants, and question arises as to the mechanism underlying inhibitory input. Phosphoinositide (PI) signaling, specifically PI3K-mediated, has been implicated in this process. For example, odorants rapidly activate PI3K in rodent olfactory cilia *in vitro*, and PI3K β and γ are present in the cilia (Ukhanov et al 2010). The detailed interaction of inhibitory odorant pairs, e.g. octanol and citral, extend this understanding. The response to octanol can be inhibited by citral, and PI3K β and γ specific blockers eliminate or strongly reduce the inhibition. Blocking PI3K does this by changing the apparent agonist strength of the otherwise non-competitive antagonist citral. The excitation evoked by citral following PI3K blockade can be suppressed by adenylate cyclase III blockers, indicating that citral also signals through the cyclic nucleotide pathway and arguing that the OR can mediate ligand-induced selective signaling (LISS). Further, screening panels of odorants representing different chemical classes, including aldehydes, alcohols, esters, aromatics, as well as selected single odorants, shows that different molecular classes of odorants that otherwise are weak or non-agonists for a particular ORN increase their agonistic strength in a PI3K-dependent manner. In contrast, odorants that are otherwise relatively strong agonists for that ORN are PI3K-independent. These findings collectively argue that PI signaling acts in concert with cyclic nucleotides in mammalian ORNs, most likely by LISS through the OR. Given that different molecular classes of odorants can function as PI3K-dependent antagonists, this mechanism offers a broad basis for opponent coding in mammalian ORNs. Supported by the NIDCD through DC001655 and DC005995.

Poster session II Poster #10

Genetics of heat-seeking behavior in the yellow fever mosquito *Aedes aegypti*

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Female mosquitoes must obtain a vertebrate blood meal to produce eggs, and they use body heat, moisture, carbon dioxide, odorants, and visual cues to locate hosts in their environment. We have established an assay to measure heat-seeking behavior in the yellow fever and dengue fever mosquito *Aedes aegypti*. We have found that female mosquitoes preferentially land and probe on warm objects in the presence of elevated carbon dioxide concentration that mimic host respiration. Thermosensitive sensilla of mosquito antennae respond to warm air and can detect minute temperature changes, perhaps allowing for thermotaxis towards a warm-blooded host.

The molecular basis of thermosensation in mosquitoes is not known, but studies in *Drosophila melanogaster* flies point towards the Transient Receptor Potential A1 (TRPA1) ion channel as a thermoreceptor activated by heat and required for thermotaxis. *Anopheles gambiae* mosquito TRPA1 is also activated by heat, and is expressed in neurons of thermosensitive sensilla. This suggests that TRPA1 may be used as a peripheral heat-sensor by mosquitoes. We have analyzed *AaegTRPA1* transcripts from *Aedes aegypti* and have identified several splice variants of this gene. To determine the role of *AaegTRPA1* in heat-seeking, we have used zinc finger nucleases (ZFNs) to generate *AaegTRPA1* null mutant mosquitoes. We hypothesize that *AaegTRPA1* is a thermoreceptor expressed in heat-sensitive neurons of thermosensitive organs, and that *AaegTRPA1* contributes to mosquito heat sensation and heat-seeking behavior. Ongoing work examining the behavioral effect of the ZFN-induced mutation on heat-seeking behavior will be presented.

Poster session I Poster #241

Long-term odor and face recognition as a function of familiarity

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This study investigated episodic recognition memory for odors and faces in the long-term as a function of familiarity. Eighty-three subjects (43 women, 40 men) encoded familiar and unfamiliar odors and faces and memory was assessed at four occasions; immediate, 4, 16 and 64 days after encoding. The results showed significant forgetting of odors and faces across time, higher overall recognition memory for faces than for odors, better retention for familiar than unfamiliar information, and no influence of gender on memory performance. In addition, hit rate performance was positively associated with naming ability at encoding, although the relationship was much less pronounced for faces than for odors. Interestingly, the decline in odor memory was primarily driven by an increment in false alarm rates over time. This observation indicates that episodic retention of olfactory information is susceptible to the passage of time.

This work is supported by the Swedish Research council to ML.

Poster session I Poster #145

Configural perception of a six-odorants mixture in newborn rabbits and human adults

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Throughout their development, mammals are exposed to complex environments. Odours from the surroundings arise from mixtures of volatiles, and even when organisms respond to key-odour cues, they respond to cues perceived among other. Several perceptual interactions may occur over mixture coding and processing (e.g. synergy/masking). Configural perception is another form of interaction. Unlike analytical perception, based on the perception of the components' odours, configural processing leads to the perception of a new odour, distinct from the odours of the components (in humans, the effect is called blending effect). Here, we evaluated the perception of a senary mixture both in human adults and newborn rabbits using in one hand (human) a sorting task to get odour resemblance results and a 3D-odour-space representation to illustrate them, and in another hand (rabbit) single-trial conditioning to confer a value to the stimuli and behavioural testing (sucking response) to quantify their perception. We used a mixture from which emerges (in humans), at a specific ratio of odorants, a red cordial odour. First, we showed that the mixture is perceived as being different from its components in both species: human subjects sorted out separately the mixture and its single components; rabbit neonates did not respond to the mixture after learning one component. Second, this difference in perception appeared for the red cordial mixture, but not for another mixture of six components: human subjects gathered this other mixture and some of its components, rabbit pups responded to the mixture. Third, the red cordial mixture was no longer perceived as a configuration after modification of certain or all the components proportion. Thus, perception of configurations in relatively complex mixtures appears not systematic but dependent on chemical composition and components proportion. The modulation of the perception presents overlap between species and between ontogenetic stages.

Poster session I Poster #197

Computational assessments of olfactory receptor odorant interactions

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Computational methods have allowed a glimpse into the mechanisms of the first step leading to olfaction. Odor molecules follow a path from outside the nostrils through the mucus membrane, bind, interact with and excite the olfactory receptor, thereby facilitating a signal transduction process that leads to our sense of smell. We have performed rigorous computational assessments of several experimentally (functionally) well characterized olfactory receptors: rat OR I7, mouse ORs S79 and S86 (part of a comprehensive combinatorial functional analysis), human ORs 17-209 (OR1G1) and the "functional pseudogene17-210 (OR1E3P).

Our computational methodology include: creating the protein model, computationally binding an odorant (experimentally shown to bind the receptor) into the OR model binding region, simulating an aqueous environment and a lipid bilayer representing the plasma membrane, and performing long-duration molecular dynamics simulation studies of the odorant behavior in the OR binding pocket. Our results have, all firsts for the field, allowed us to view in real time entry and exit paths from and to the binding region of the OR. We have also observed how specific residues contribute to odorant binding—a combination of van der Waals and electrostatic interactions—over the course of a simulation. We can observe the involvement of specific amino acid residues from the inter-helical loops. We have been able to show a strong correlation between excitation of an OR by an odorant and preferential binding between two binding regions of an OR. It has long been surmised that OR excitation proceeds from a structural change following OR-binding. We have been able to pinpoint this structural excitation in the OR.

Poster session II Poster #242

Sex differences in orienting reaction towards olfactory cues

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Women typically outperform men in olfactory tests. They exhibit higher olfactory sensitivity higher olfactory identification abilities and superior memory for familiar odors. Further more, olfaction seems to be more important to women compared to men. Now, we also found evidence for sex differences in orienting reaction towards odors.

60 women and 59 men were presented to three aversive and one neutral stimulus offered through different sensory channels: vision, odor and audition. During stimulus presentation heart rate and skin conductance were obtained and participants were asked to rate both pleasantness and intensity of the stimuli.

Similar patterns of autonomic response were found in both sexes for visual and auditory stimuli. However, for olfactory presentation only men exhibited the typical initial heart rate deceleration, women showed almost no change of heart rate. The effect could be modulated by different stimulus qualities. 'Environmental rejection' is presumably reduced in women mirroring the fact that women require less attention for categorizing odors.

Poster session II Poster #198

Neural coding of binary mixtures in a structurally related odorant pair

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Evidence suggests that the encoding of odorant mixtures by olfactory sensory neurons is influenced by molecular interactions at receptors. However, it is unclear whether mixtures evoke overall patterns of receptor activation governed primarily by linear summation, or by non-linear mixture interactions. We analyzed inputs to olfactory bulb glomeruli evoked by binary mixtures of a pair of structurally similar but perceptually distinct odorants, eugenol and methyl isoeugenol. Fluorescence imaging of glomeruli in synaptopHluorin mice revealed highly overlapped activation of receptor populations, increasing the likelihood of mixture interactions. In overlapped glomeruli, we found approximately linear summation of inputs in a concentration-dependent and reciprocal manner up to response saturation. Observed compression of responses from mixtures was predicted by simulations based on a well-fitted, modified Hill equation model of competitive agonism at receptor binding sites. Model fits did not require either non-competitive receptor interactions, or spatial segregation of activity in the nasal epithelium.

Poster session II Poster #148

Anterior piriform cortex activity induced by in vivo optical stimulation of the olfactory bulb

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Odor stimuli elicit spatio-temporal patterns of neuronal activity in the olfactory bulb (OB). To date it remains open which features of these patterns are decoded by cortical neurons. To address this we activated individual functional modules in the olfactory bulb, defined as group of mitral and tufted cells (M/Ts) belonging to a single glomerulus using optical stimulation of M/Ts expressing Channelrhodopsin-2 from the Thy1 promoter [Arenkiel et al. Neuron, 2007]. We tested such transgenic mice *in vivo* under urethane anesthesia using blue light stimulation of the dorsal OB surface and juxtacellular recordings from single neurons in the ipsilateral anterior piriform cortex (aPCx). Patterns of blue light (465 nm, 6-10 mW/mm2) were projected onto the OB surface using a custom-built system based on a digital mirror device. Sparse stimuli of variable size (20 ms duration & respiration triggered) or dense white noise stimuli (spot size ~60 x 60 µm, on probability 10%, refresh rate 50 Hz) were used to find locations activating or inhibiting individual aPCx neurons. Individual aPCx neurons could only be driven by stimulation of extended areas (> 200 μm^2) of the OB surface covering several glomeruli. Stimulation efficacy depended on stimulus size as well as on stimulus location. Spike latencies were longer compared to those of M/Ts. In most cases effective stimuli not only increased the firing rate but also resulted in a prominent reduction of spontaneous activity after the end of the stimulus. Despite a significant increase of firing rate of aPCx neurons during dense noise stimulation, spike triggered averaging analysis rarely revealed specific patterns of OB activity. In summary, our data are consistent with the notion that several glomerular modules have to be coactive to effectively drive cortical neurons and that the stimulation efficacy of individual patterns varies substantially.

Poster session I Poster #415

Highly sensitive detection of ammonia using solution processable polyaniline/carbon black composite sensors

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A highly sensitive ammonia sensor has been fabricated by depositing solution processable polyaniline/carbon black conductive composites on to flexible plastic substrates with gold interdigitated electrodes. Polyaniline (PANI) is known to be an excellent sensing material for ammonia, since NH_3 deprotonates the amine group in emeraldine salt (doped form of PANI), converting it to emeraldine base (undoped form) which may cause a significant decrease in conductivity. The very high affinity of PANI to ammonia has its drawbacks, mainly with regard to sensor recovery where long timescales are required to return the sensor to its baseline. Operation at elevated temperatures can improve desorption of NH_3 molecules from the sensor film, hence improving the recovery time.

Unfortunately, lack of solution processability, the inherent problem with conventional PANI especially in its doped stat, has limited implementation of this material in conventional sensor fabrication methods. Here, we have exploited sulphosuccinic acid (SSA), a multifunctional dopant with sulphonic and carboxylic acid groups, which not only renders the doped PANI soluble in aprotic solvents such as n-methyl pyrrolidone (NMP), but also makes it possible to prepare conductive composites of PANI/carbon black (CB) simply by mixing surface modified carbon black with PANI solution. Sensors were fabricated by depositing a thin layer of PANI/CB dispersion in NMP via dip coating on flexible polyimide and polyethylene naphthalate substrates incorporating a heater.

Sensor response to very low concentrations of ammonia vapour at 80 ˚C was measured. Due to high affinity of polyaniline to ammonia and presence of conductive pathways of carbon black through out the composite layer, the sensor exhibits sensitive and fast response down to ppb concentrations. The high sensitivity, rapid response and good reversibility of the sensor make it promising for real-time ammonia sensing applications.

Poster session II Poster #12

Changes in peripheral olfactory system mediate preference for different host in *Drosophila mojavensis*

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Olfactory perception is a primary means by which many animals gain information about their surrounding environment, from recognition of appropriate food and oviposition sites to the modulation of social interactions. Divergence in these olfactory preferences as a result of local adaptation to different ecological environments has been well documented and can contribute to reproductive isolation among populations. An understanding of the underlying genetic mechanisms, however, is more limited. Here, we examine host specialization in the cactophilic fly, *Drosophila mojavensis* that specializes on four different host cacti, each in a different part of its range. We show that *D. mojavensis* is attracted to a specific stage of fermenting cactus and a particular cactus-specific volatile composition. We also show that there is divergence in electrophysiological responses for one of the four *D. mojavensis* subspecies to select cactus volatiles. Behavioral variation among subspecies was also observed in response to individual cactus volatiles and as well as to mixtures. Finally, we assess changes in the *D. mojavensis* olfactory system through whole transcriptome sequencing. We observed greater than 2-fold expression difference for several odorant receptors and odorant binding proteins. These results identify olfactory genes, which may contribute to host-specific adaptation and ultimately lead to reproductive isolation in *D. mojavensis*.

Poster session I Poster #13

Disrupting mosquito attraction to host cues by targeted mutagenesis of the orco olfactory co-receptor

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Human host odor is a long-range, attractive cue that guides mosquitoes to their hosts. The mosquito perceives differences in host odor, both between and within species, to determine which host to feed upon. The molecular mechanism by which mosquitoes translate host odor information into host-seeking behavior has been inferred but not demonstrated. Insects use two families of olfactory receptors, the Ionotropic Receptor (IRs) and the Olfactory Receptors (ORs), to detect volatile chemicals in their environment. To shed new light on mosquito olfactory-driven behaviors, we developed a technique for targeted mutagenesis in *Aedes aegypti* using zinc-finger nucleases (ZFNs) and proved that it can work by heritably disrupting GFP. To understand the role of ORs in mosquito behavior, we targeted *orco*, a gene formerly known as *Or83b* in *Drosophila* and Or7 in mosquitoes, which is an obligate co-receptor for ORs. We demonstrate that *Aedes orco* is necessary for electrophysiological responses to 1-octen-3-ol but not to CO_2. We are currently using olfactometer-based assays to understand how *orco* null mutant mosquitoes respond to human and non-human host odors, chemical repellents, and plant volatiles. The establishment of loss-of-function genetics in *Aedes aegypti* will open new paths of investigation in the genetics of vector biology.

Poster session II Poster #14

Neural and molecular correlates of a polymorphism in pheromone preference in the European corn borer

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Attraction of male moths to female-released pheromone is an exemplar of olfactory acuity. Exclusive communication channels at the species level rely on distinctive components and blends, often in highly specific ratios. The response of males to pheromones are accordingly robust and highly specific, which offers excellent opportunities to study network correlates of olfactory preference.

Males of the crambid moth, *Ostrinia nubilalis*, the European corn borer, are particularly interesting to study olfactory preference. Males are narrowly tuned to the ratio of binary blend of female-released Z11-14:OAc and E11-14:OAc. A naturally occuring pheromone polymorphism exists in field populations of the European corn borer. Two strains exist, the Z and the E strain, which produce and prefer opposite ratios of Z11-14:OAc and E11-14:OAc, 97:3 and 1:99, respectively. Hybrids, although rarely encountered in the field, produce and prefer intermediate ratios. A single sex-linked locus underlies this difference in preference. Here we present data on how this major swap in preference is mediated through changes in the olfactory circuitry, both in the periphery, through expression patterns of olfactory receptors, and in the brain, at the level of the antennal lobe.

Poster session I Poster #15

What reaches the antenna?How to calibrate odor flux and ligand-receptor affinities

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Physiological studies on olfaction frequently ignore the airborne quantities of stimuli reaching the sensory organ. We used a GC-calibrated photoionization detector (PID) to estimate quantities released from standard Pasteur pipette stimulus cartridges during repeated puffing of 27 compounds, and verified how lack of quantification could obscure olfactory sensory neuron (OSN) affinities. Chemical structure of the stimulus, solvent, dose, storage condition, puff interval, and puff number all influenced airborne quantities. Unlike vapor pressure, a model containing boiling point and lipophilicity predicted airborne quantities of compounds in paraffin reasonably well.

We recorded OSN responses of *Drosophila melanogaster*, *Ips typographus*, and *Culex quinquefasciatus*, to known quantities of airborne stimuli. These demonstrate that inferred OSN tuning width, ligand affinities and classification can be confounded and requires stimulus quantification. Additionally, proper dose-response analysis shows that *Drosophila* AB3A OSNs are not promiscuous, but highly specific for ethyl hexanoate, with other earlier proposed ligands 10 to

10000-fold less potent. Finally, we reanalyzed published *Drosophila* OSN data (DoOR) and demonstrate substantial shifts in affinities after compensation for quantity and puff number.

We conclude that consistent experimental protocols are necessary for correct OSN classification, and present a few simple rules that will make calibration, even retroactively, readily possible.

We hope to be able to demonstrate the RAE PID "in vivo" at the poster. Welcome!

This work is in press as: Andersson MN, Schlyter F, Hill RS, Dekker T (2012). What reaches the antenna?How to Calibrate Odor Flux and Ligand-Receptor Affinities. Chemical Senses 37.

Poster session I Poster #243

Better the devil you know? Olfactory preferences and mere exposure to odors

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The more familiar an odor, the more pleasant it is judged. This positive correlation between the dimensions of familiarity and pleasantness of smells seems to constitute a reliable and unchallenged result in olfactory research. This relation between familiarity and pleasantness has been mainly explained by the mere exposure effect - a psychological theory claiming that the repeated exposure to a stimulus is sufficient for the enhancement of one's attitude toward it - i.e. to induce a subjective preference increase. In this talk, we will present a series of experiments that question the validity of this relation, on the basis of several empirical data and from both theoretical and methodological points of view. First, we will present correlational studies highlighting that the relation between pleasantness and familiarity is specific to pleasant odors, on the basis of subjective ratings and psychophysiological measures. Then, we will report cross cultural studies confirming the weakness of familiarity influence on the perception of malodors around the world. In a last experiment, we directly manipulated the familiarity of odors by presenting different unpleasant, neutral and pleasant odors repeatedly during 6 consecutive days. Results showed a strong increase of preferences for the mildly pleasant / neutral odors (mere exposure effect) but significantly less increase in pleasantness for both malodors and very pleasant odors. Taken together, this streamline of data underline the limits of preference modulation through mere exposure. The results will be discussed in light of the biological relevance of odors for individual survival.

Poster session II Poster #16

Natural variation in cuticular hydrocarbons in the *Drosophila* Genetic Reference Panel

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Cuticular hydrocarbons (CHCs) act as close range and contact pheromones in many insects and are a critical factor in *D. melanogaster* mate choice. Variation in CHCs can potentially alter mate choice in natural populations leading to assortative mating and incipient reproductive isolation. The *Drosophila* Genetic Reference Panel (DGRP) is a panel of 192 inbred lines of *D. melanogaster* derived from a natural population at the Raleigh NC Farmer's Market. Complete genome sequences are available for the lines and enable genome-wide association (GWA) analysis to identify genes that harbor polymorphisms associated with variation in CHCs and/or mating behavior. Thus, the DGRP presents an opportunity for an unbiased approach to uncover the genetic basis contributing to natural variation in CHCs present on both males and females. GWAs with the DGRP have the power to detect variants with moderately small to large effects on complex CHC composition. We collected gas chromatography spectra of flies from the DGRP in both sexes and quantified relative abundance levels of CHC components, as well as the total amount of CHCs. A majority of the CHCs show significant variation, including 7,11-heptacosadiene, the predominant female pheromone. We are using 2.5 million single nucleotide polymorphisms from whole genome sequences to associate differences in CHC composition with variation at genomic loci. In addition, variation in CHC composition can be correlated with other traits such as longevity, mate choice, and aggression. These results may offer insight into the link between variation in pheromone composition with mating behavior and incipient speciation.

Symposium 3 "Chemosensory receptors in non-chemosensory tissues" Saturday 23 June

Nutrient sensing of the ghrelin cell

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Obesity is one of the major healthcare problems of our modern society reaching epidemic proportions. To prevent nutrient excess, the body relies on nutrient sensors that detect nutrient availability and coordinate effectors of energy intake and utilization. One of these effectors is ghrelin, a 28 amino acid octanoylated peptide, produced by the stomach involved in the regulation of energy –and glucose homeostasis. Plasma ghrelin levels increase before each meal and return to basal levels after the meal to dictate the timing of the meals. The postprandial ghrelin suppression is dependent on the macronutrient composition of the meal suggesting that the ghrelin cell may sense nutrients. Recent studies have identified taste receptors, not only in the lingual system but also in the gastrointestinal tract where they function as chemodetectors for intestinal nutrients. We found that ghrelin cells are co-localized with the gustatory G-proteins, α-gustducin and -transducin. Intragastric administration of bitter compounds increased plasma octanoyl ghrelin levels. The effect was partially mediated via -gustducin and resulted in a temporary increase in food intake. This was followed by a long-term decrease in food intake which correlated with an inhibition of gastric emptying. Our studies also showed that food is an important source of fatty acids for ghrelin and that α-gustducin is involved in the fatty acid sensing cascade of the octanoylation process. Nevertheless a role for the free fatty acid sensing receptors GPR40 and GPR120 seems unlikely.

Our studies show that the ghrelin cell can sense bitter compounds and fatty acids via interaction with gustatory signaling pathways in the gut. Since the expression of gustatory-signaling elements is altered in the stomach of obese patients, targeting of nutrient sensors on endocrine cells in the gut could be considered as therapeutic targets for the treatment of obesity.

Poster session I Poster #151

Modulation of basal forebrain neural activity in the rat during spontaneous and reward-motivated olfactory behavior

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In the mammalian olfactory system, ascending sensory information is integrated with an extensive network of descending projections from cortical and neuromodulatory structures. In particular, the olfactory bulb and piriform cortex are targeted by inputs from the horizontal limb of the diagonal band of Broca (HDB), part of the cholinergic basal forebrain (BF) complex. Cholinergic modulation serves a critical role in regulating olfactory perception (Fletcher and Chen, 2010), although, to date, little is known about the dynamics of BF inputs to the olfactory system during behavior. To investigate the relationship between olfactory behavior and BF neural activity, we obtained chronic extracellular recordings from neurons in the HDB of awake, unrestrained rats engaged in spontaneous and reward-motivated olfactory behaviors. In general, HDB neurons were tonically active in the waking state [mean firing rate: 14.02±14.29 spikes/sec, n=30]. In a spontaneous odor investigation task, we observed a population-level significant increase in firing rate ($p=0.015$) during active odor sampling. Moreover, a substantial fraction of neurons (6/18) exhibited rhythmic spiking activity in the respiratory theta (5-8 Hz) range. To investigate neural dynamics during reward-motivated learning, we engaged rats in a forced-choice odor discrimination task and analyzed activity during four task-related epochs (baseline, odor approach, odor sampling, reward selection). Firing rates of a majority of HDB neurons (9/12) changed significantly relative to baseline during at least one epoch. Furthermore, in 42% of neurons, baseline firing rates decreased significantly during performance of post-criterion, as compared to pre-criterion, trials. Together, these results suggest that BF inputs to the olfactory system are not only active but are dynamically regulated during behavior. Research supported by NIH NIDCD grants DC010420 (CL and DMS) DC011974 and a L'Oreal USA For Women in Science Fellowship (SD).

Poster session II Poster #152

Epigenetic marking of an olfactory receptor in the mouse olfactory epithelium – potential implications for learning-induced structural and functional plasticity.

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The perception of a sensory stimulus and how it is processed within the central nervous system are crucial components underlying behavioral output to the stimulus. Using a classical conditioning paradigm, we can ask how the olfactory system responds to an odor after adult male mice have been trained to associate odor presentation with a mild foot-shock. Previous work in the Ressler laboratory has shown that pairing presentations of Acetophenone (ACE) with mild foot-shocks results in increased numbers of ACE-responding Olfactory Sensory Neurons (M71-OSNs) in the main olfactory epithelium (MOE), and increased M71 glomerular volume in the olfactory bulb. No such observations were made when animals were conditioned with Propanol (PROP), an odor that does not elicit responses from M71 OSNs. One possible mechanism to explain these observations is enhanced M71 olfactory receptor (OR) choice in the MOE after conditioning. Recent data suggest that histone modifications around OR loci play a critical role in determining OR choice. In the current study, we seek to examine an epigenetic basis for the increased M71 OSN number and M71 glomerular volume after conditioning. To this end, we have performed N-ChIP on whole MOE of ACE- and PROP-conditioned adult male mice, followed by qPCR for the M71 olfactory receptor. Compared to PROP-conditioned males, ACE-conditioned animals have significantly more M71 DNA associated with the acetylation of Histone H3, a modification considered permissive to transcription. In contrast, we do not observe any differences between ACE and PROP groups when DNA is immunoprecipitated with antibodies that recognize other histone modifications that are either permissive (AcetylatedH4K12, H3 trimethylK4) or repressive (H3 trimethyl K9, H3 trimethyl K20) to transcription. This observation leads us to suggest that an epigenetic mechanism accompanies the learning-induced structural plasticity observed in the olfactory system after classical conditioning.

Poster session I Poster #153

Olfactory signal transduction in phosphodiesterase 1C knockout mice

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In mammalian olfactory receptor neurons (ORNs) signal transduction begins with the binding of an odor molecule to odorant receptors (ORs), which in turn, via a G protein-coupled cascade, activate adenylyl cyclase III (ACIII) and increase intracellular cAMP. cAMP opens the cyclic nucleotide-gated (CNG) channel to initiate odorant-induced depolarization. Two phosphodiesterases (PDEs) are expressed in ORNs, one located in the cilia (PDE1C) and one restricted to the dendrite and cell body (PDE4A). Both are able to decrease cAMP after odorant stimulation. Also, in the absence of stimulation, basal activity of ORs drives basal fluctuations of cAMP, which is observed as baseline current noise that leads to basal action potential firing. The varying noise levels associated with each OR type differentially affect ORN physiology and, thus, odorant-response kinetics.We investigated the role of PDE1C in ORN physiology by using the suction pipette technique to record from ORNs dissociated from PDE1C knockout mice that had been crossed with odorant receptor-tagged mice (mice expressing GFP in ORNs expressing the I7 OR or mEG-OR).

We found little difference in peak amplitude and time course in eugenol responses in the mOR-EG ORNs regardless of whether PDE1C was present or not. In contrast, in I7 ORNs the response magnitude to heptanal was greatly reduced and the time for the response to reach its peak magnitude was prolonged once PDE1C was knocked out. We also studied the odorant dose dependency of mOR-EG and I7 ORNs. Interestingly, PDE1C does not seem to control the overall sensitivity of ORNs since no shift in the dose-response relation was observed in I7 and mOR-EG ORNs. Instead a reduction of the I7 ORN dose-response at higher odorant concentrations was observed, which was not seen in mOR-EG ORNs.

Together our results suggest that PDE1C is important to retain response magnitude and kinetics across ORNs expressing different ORs.

Poster session II Poster #100

Olfaction in the Red Flour Beetle *Tribolium castaneum*

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As a member of the largest insect order and a pest of stored agricultural products, the red flour beetle *Tribolium castaneum* presents an attractive emerging model organism to study olfaction. We used comparative transcriptome analysis of antennae, legs, heads and bodies of males or females to identify tissue and sex specific expressed odorant binding proteins (OBPs) and odorant receptors (ORs). Based on our preliminary data we got evidence for 84 ORs, 13

OBPs/C-OBPs and 2 chemosensory proteins (CSPs) enriched in adult antennae. We identified 3 OBPs that are expressed at least three fold higher in females than in males and 2 C-OBPs who are more abundant in males, respectively. These proteins are good candidates for pheromone binding proteins and are currently analyzed in more detail. Using the RNAseq data we also revised the predicted ORF of OBPs, C-OBPs, and CSPs, to be able to calculate the correct peptide masses and compare them to MALDI-TOF mass spectrometry data obtained from the antennae to confirm them on protein level. We used different molecular techniques, both descriptive (*in situ* hybridization and immunohistochemistry) and functional approaches such as RNA interference to knock down genes and transgenic techniques for ectopic- and misexpression. Based on these approaches, we are currently developing optogenetic tools to further analyze OBP and OR functions in the red flour beetle. This study was funded by the DFG Schwerpunktprogramm - SPP 1392 `Integrative Analysis of Olfaction´.

Poster session II Poster #360

Lingual inhibition of serotonin re-uptake has differential effects on sweet, salt and bitter taste modalities in humans.

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Systemic serotonin re-uptake inhibition using serotonin-reuptake inhibitors (SSRI) enhances both sweet and bitter taste after 2 hours (1), but in isolated taste cells, serotonin re-uptake inhibits Type II (receptor) cells (2). We previously showed that application of SSRI to the tongue acutely raised bitter taste thresholds (3). SSRI are themselves bitter tasting compounds however, and this observation may reflect bitter adaptation rather than an effect of serotoninergic modulation on taste buds per se.

36 (17 male, 19 female, age range 18-51 years) participants gave informed consent for inclusion in the study. Bitter thresholds (n=21), sweet (n=15), and salt (n=36) thresholds and intensities were determined. Different concentrations of sweet (sucrose), bitter (quinine hydrochloride) salt (NaCl) solutions were applied to the tongue in a pseudorandom order before and 15 minutes after drug (paroxetine) or placebo also applied directly to the tongue. All protocols were approved by the Faculty of Medical and Veterinary Sciences Ethics committee, University of Bristol.

SSRI caused a significant increase in bitter threshold (98±71μM) vs placebo (- 25±17μM), a significant decrease in sweet threshold (5±9mM) vs placebo (49±20mM) and no change in salt (10mM±4mM SSRI vs. -7±4mM pl). At 60min there was no change in sweet and a decrease in salt threshold. There was no effect on whole mouth sweet or salt taste intensity over the same time. The differential effect of lingual SSRI on taste thresholds, with no effect on taste intensity, suggest that the effects of SSRI on taste thresholds are a drug effect, rather than adaptation as a result of topical bitter drug. These data show that acute (minutes) inhibition of 5-HT reuptake at the taste bud inhibits bitter taste (increases threshold), enhances sweet taste (decreases threshold) with no effect on salt taste.

(1) Heath et al J Neurosci 2006
(2) Huang YA et al J Neurosci 2009
(3) Donaldson LF et al Chem Senses (Abstr) 2008

Poster session I Poster #199

Regulation of epitheliopoiesis by Neuregulin1/ErbB signaling in the olfactory epithelium

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The olfactory epithelium (OE) is unique in its ability to undergo regeneration of all cell types throughout mammalian life in response to both natural cell turnover, as well as environmental or experimental injury, for example, by selective olfactotoxins, like methyl bromide (MeBr). Both globose basal cells and horizontal basal cells have been identified as progenitor populations responsible for regeneration, however the cues that regulate stem cell activation and function are poorly understood. The olfactory epithelium is underlain by a mesenchymal compartment, the lamina propria (LP), which, by analogy to other tissues, is a potential source of molecular signals that direct stem and progenitor cells towards tissue repair and homeostasis. We have found that the ErbB receptor family binding protein, Neuregulin1 (Nrg1), is secreted by LP cells. Moreover, Nrg1 is capable of inducing epithelial assembly in a 3D tissue culture model of the OE, developed in our lab. We have identified a specific isoform of Nrg1, Nrg1 Type III beta 1 a, as the predominant isoform in the olfactory mucosa, and using a recombinant protein, we have been able to show that the beta domain of Nrg1 is capable of stimulating sphere formation, and thus stem cell differentiation, in our 3D tissue culture model. In addition, we have used a knock-out mouse for the Nrg1 receptor, ErbB4, to show that loss of downstream signaling results in an aneuronal phenotype and the inability to recover after MeBr injury. Together, these studies provide novel insight on the effects of a mesenchymal-secreted protein, Nrg1, on OE differentiation, and implicate a well-known developmental signaling pathway in OE differentiation.

Symposium 20 "Aquatic olfaction" Tuesday 26 June

Neural pathways visualized by odour –specific endocytosis in the olfactory organ in fish.

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The sensory neurons in the olfactory epithelium display an extensive endocytosis (Bannister and Dodson, Microsc Res Tech 1992, 23: 128-141) as a part of the constitutive endo-exocytotic cycle. Recently, we have shown that there is a ligand-specific endocytosis in addition to the constitutive endocytosis, which makes it possible to stain a particular set of sensory neurons (Døving et al., J Exp Biol 2011, 214: 80-87). As marker dyes of the ligand-specific uptake from the external milieu, we have used different dextrans conjugated with Alexa dyes. The dextrans internalized by endocytosis, are visualized in the sensory neurons, they are transported across the cell soma and further along the axons towards the olfactory bulb where they terminate in the glomeruli. Given sufficient survival time, the dye pass the synapses and stain secondary neurons of the olfactory bulb in crucian carp. These findings makes it possible to stain the neural pathways related to a particular odour released behaviour. The advantages and shortcomings of this method will be dis-cussed.

Symposium 9 "Chemosensory initiated mating behaviour" Sunday 24 June

The olfactory mediation of mate choice in lemurs

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Sexual selection theory is traditionally modeled according to additive genetic benefits or good genes, whereas more recent models invoke genetic compatibility or good fit. If maintaining genetic diversity positively influences fitness, advantages would accrue to animals that could decipher honest indicators of genotype in potential mates. There is increasing evidence to suggest that the signaling of both inherent quality and relative compatibility could be mediated by olfactory cues, such that animals could trade-off between models to optimize their mate choice. Using an integrative research program (merging genetic, chemical, endocrine, and behavioral approaches), we study olfactory-guided mate choice in various Malagasy primates. Their unusual behavioral ecology and olfactory reliance make them ideal models for comparative study. Chemical analyses of glandular or urinary volatiles confirm that odorants often differ by the same reproductive, demographic, and genetic variables that influence olfactory behavior. For example, lemurs in breeding condition have richer scent chemistry than nonbreeding or contracepted lemurs and generate more interest from conspecifics in a 'signaler-receiver' paradigm. Sex-role reversal also may be signaled via odor, as female-dominant lemurs display more elaborate scent glands and express more chemically complex odors than do males. Lastly, we estimate genome-wide neutral heterozygosity and characterize functional diversity at the Major Histocompatibility Complex to test for odor-gene covariance: To date, we have linked genetic diversity to chemical diversity, showing honest olfactory ornamentation in both sexes. Lemurs detect both genetic relatedness and genetic diversity from the scent of unfamiliar conspecifics. Beyond supporting an olfactory mechanism of condition-dependent signaling in a primate, we suggest that animals could rely on conspecific olfactory cues to distinguish information about good genes *and* good fit. Funded by NSF.

Delwart Contributed Symposium - Higher olfactory processing Tuesday 26 June

A microRNA-Dopamine receptor genetic regulatory module in distinct neural circuits for olfactory arousal and olfactory memory.

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The ability form olfactory associative memories requires circuit mechanisms to integrate accurate and specific odor

representations with aversive or appetitive unconditioned stimuli (USs). The US signal can include information about valence, intensity and quality of the stimulus, all of which may modulate the arousal state of the animal. We will describe a micro-RNA::dopamine receptor genetic regulatory module that plays distinct and separable roles in olfactory arousal on the one hand and on odor-US association on the other. MicroRNA genes do not code for proteins, but instead generate small noncoding RNAs that regulate expression of specific target mRNAs with complementary target motifs. Although a growing number of studies demonstrate that micro-RNA function broadly speaking plays an important role in the brain, there still are few examples where function of individual micro-RNAs have been connected through neural circuits to behavior. We demonstrate that normal olfactory behavior in *Drosophila* requires the micro-RNA dme-miR276a in two different neuronal cell types: mushroom body Kenyon cells and ellipsoid body R4 central complex neurons. In both circuits, miR276a targets a DA1 type dopamine receptor. But this regulatory relationship serves different aspects of olfactory behavior in mushroom body versus central complex. This miR276a-Dopamine receptor interaction in mushroom body neurons plays a role in long-term olfactory associative memory per se and in ellipsoid body neurons the same regulatory interaction modulates olfactory arousal.

Poster session II Poster #246

Olfaction-pain interactions: A familiar odor enhances pain tolerance

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Previous research suggests that odors, in particular pleasant and sweet ones, enhance pain tolerance (Marchand & Arsenault, 2002; Prescott & Wilkie, 2007). In neonates the familiarity of an odor is instrumental in lowering pain responses (Goubet et al., 2007). The current study examined the impact of a non-sweet familiar odor on pain tolerance in women. First, all participants (N= 62) watched a short movie. Half the sample was unknowingly exposed to an odor while the other half was not. Participants were then asked to immerse their hand in cold water for as long as they could tolerate (cold pressor test, CP) while being exposed to an odor or no odor. Participants were randomly assigned to one of four conditions: Odor familiarization/same Odor during CP (O/O, n = 16), Odor familiarization/No Odor during CP (O/N, n = 15), No odor familiarization/Odor during CP (N/O, n = 16), No odor familiarization/No odor during CP (N/N, n = 15). Measures included pain perception at immersion, 20 s later, and at removal, mood ratings before and after the CP test, and time spent in water. Pain perception increased in all groups, $F(2, 116) = 84.97$, $p < .01$, with pain being most acute at removal, ps. $< .01$. Mood was significantly lower after the CP test, $F(1, 58) = 33.94$, $p < .01$. Participants in the O/Odor group test kept their hand in water significantly longer (M = 112.2 s) compared to participants in the O/N and N/N groups (M = 59.11; M = 55.67), ps. $< .05$. Participants in the N/O group showed no increase in tolerance (M = 75.14) compared to the other groups. These results suggest that pain tolerance can be enhanced via the presentation of a familiar odor. This effect could be supported by the re-encounter of an olfactory cue previously associated with a pleasant context (watching a movie). Results are discussed within the context of the importance of familiarity in emotional regulation.

Poster session I Poster #17

On the origin of insect olfaction

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The evolutionary predecessors of insects left the aquatic environment about 400 million years ago. Such a transition is highly dramatic for sensory function. Instead of detecting water-soluble compounds, the olfactory system had to register airborne, often hydrophobic molecules. Another dramatic event in the evolution of insect sensory systems was the emergence of flowering plants as a new resource. One way to reach conclusions regarding the evolutionary processes that have shaped the exquisite olfactory system of present day advanced insects is to study their primitive cousins. Preciously

little is, however, known regarding the olfactory capabilities of these most basal insects. Here we describe the peripheral olfactory sensory basis for odor detection and discrimination in primitive insects ranging from the ancient apterygotan bristletail *Lepismachilis y-signata* (Archaeognatha: Machilidae), via the firebrat *Thermobia domestica* (Zygentoma: Lepismatidae), the paleopteran dragonfly *Sympetrum sanguineum* (Odonata, Libellulidae), to the neopteran leaf insect *phyllium siccifolium* (Phasmatodea: Phyliidae). As a comparison we perform the same study in the model insect *Drosophila melanogaster*. We systematically characterize olfactory sensory neurons (OSNs) innervating olfactory sensilla on the antennae of these animals physiologically, using single sensillum recording technique (SSR) and an odorant set composed of volatiles belonging to diverse chemical classes. We characterize OSN responses by determining tuning width, response dynamics, odor sensitivity and odor space representation. We also use machine learning algorithms, (Naïve Bayes assignment tests) to predict whether the OSNs studied in the non-model insects adhere to properties expressed by *D. melanogaster* members of the canonical insect odorant receptor (OR) family or of the recently discovered iontropic receptor (IR) family. From our results we conclude that the OR-based olfactory system did most likely not evolve as a result of the transition to land, as basal insects seem to express only the IR-based system. Higher insects, on the other hand, possess both systems, possibly as an adaptation to life on flowering plants. In parallel to our studies of the periphery, we reconstruct the primary olfactory centers, the antennal lobes, of the apterygotan firebrat and the neopteran leaf insects. From these studies we address issues as to the evolution of the antennal lobe from basal to higher insects, and how the organization of this center matches adaptations observed in OSNs.

Poster session I Poster #319

Psedogenization of the umami and sweet receptor genes in cetacean

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Everyone has her or his likes and dislikes for food, but few people think it is due to heredity even in part. Many reports have been published describing polymorphisms such as SNPs associated with taste perception or food preference, but most of them failed to address it. It could be thought to be because SNPs have weak association with taste preference. In this study we focused on taste preference of mammals except experimental animal such as mouse or rat. Roughly speaking, mammals are divided into two groups, which are carnivorous and herbivorous animals. They choose their food as they like, in other words, “instinctively” or “genetically.” Here we report comparative analysis of umami receptor gene, *T1R1* and *T1R3*, and sweet receptor, *T1R2*, derived from both carnivorous and herbivorous animals, especially much about cetacean. Frameshift and/or nonsense mutations are accumulated on umami and sweet receptor genes, *T1R1*, *T1R3* and *T1R2* from four different whales and dolphins, resulting in inactivation of umami and sweet receptors. Cetaceans were originally land animals, but for some reason, they come back to the ocean. At that time, functional taste receptors might be mutated, because it might be too tasty for them in sea water. We are also discussing other mammals in sea water and fresh water.

Poster session II Poster #154

NMDA-receptor dependent synaptic activation of TRPC channels in olfactory bulb granule cells

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TRPC channels are widely expressed throughout the nervous system including the olfactory bulb where their function is largely unknown. Here we describe their contribution to central synaptic processing at the reciprocal mitral cell-granule cell microcircuit, the most abundant synapse of the mammalian olfactory bulb. Suprathreshold activation of the synapse

causes sodium action potentials in mouse granule cell dendrites and a subsequent long-lasting depolarization linked to a global postsynaptic calcium signal recorded with two-photon laser scanning microscopy. These signals are not observed after action potentials evoked by current injection in the same cells. Both depolarization and Ca^{2+} rise require NMDA receptor activation, since they are blocked by APV, and the long-lasting depolarization was entirely absent from granule cells deficient for the NMDA receptor subunit NR1. Long-lasting depolarization and Ca^{2+}rise are also absent in granule cells from mice deficient for both TRPC channel subtypes 1 and 4, whereas the individual deletions of *TRPC1* or *TRPC4* show only a partial reduction of the LLD. We confirm the presence of TRPC1 and TRPC4 protein immunhistochemically, also at the ultrastructural level, in granule cell somata and the external plexiform layer, where the synapses between mitral cells and granule cells are located. As to the synaptic function of TRPC channels at this synapse, recordings from mitral cells in the double-deletion reveal a reduction of asynchronous neurotransmitter release from the granule cells during recurrent inhibition. Thus TRPC channels contribute to the slow time course of dendrodendritic inhibition.

We demonstrate a direct involvement of TRPC channels in synaptic excitation in higher sensory processing, and provide evidence for a novel, NMDA receptor-mediated mechanism of activation of TRPCs that also affects neurotransmitter release from granule cell dendrites.

Poster session II Poster #18

Unraveling the secrets of a life below ground - Olfaction and taste in Scarab larvae

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Below ground, in the absence of light, chemosensation is of particular importance and locomotion is costly. Root-feeding *Melolontha melolontha* larvae (Coleoptera, Scarabaeidae) are exposed to gradients of airborne and water-soluble stimuli from their surrounding matrix. Historically, CO_2 was the only identified attractant in many rhizophagous species.

Here we fill a general gap of knowledge on the ultrastructure of chemosensory organs, and their physiological and behavioural responses to relevant substances in immature, soil dwelling Scarabaeids. We present a complete atlas of sensory organs on the larval antennae, maxillary and labial palps based on scanning and transmission electron microscopy. Physiological responses were recorded to more than 50 volatile compounds occuring in the rhizosphere of potential host plants like dandelion (*Taraxacum officinale*), or that are behaviourally relevant to other soil-inhabiting species. Active compounds were subsequently evaluated for their behavioural relevance. Additionally, behavioural responses to root derived sugars were tested.

Olfactory and contact-chemoreceptors were found on all examined appendages. Hygroreception is located on the palps, whereas only the antennae detect CO_2. Various alcohols, acids, amines, esters, aldehydes, ketones and monoterpenes elicit responses in antennae and palps. Electrophysiological and behavioural responses are specific to the level of enantiomers of the same compound. In behavioural assays propionic and malic acid, benzaldehyde, (-)-limonene and γ- terpinene are deterrent, while 1-hexanol, α-pinene, (+)-camphene, acetone and disaccharides attract the larvae. The obtained morphological, electrophysiological and behavioral evidence indicates that chemosensory capacities have been highly underestimated in soil-dwelling Scarabaeid larvae. Cues other than CO_2 contribute to larval orientation and, most probably in an interaction between gustation and olfaction, to host root finding below ground.

Symposium 13 "Plasticity and modulation in olfactory systems - Linnaeus Symposium" Monday 25 June

Neuromodulatory effects in the olfactory epithelium depend on odorant and physiological state

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The terminal nerve extends between the nasal cavity and basal forebrain. Its fibers contain a number of neuromodulatory compounds, including gonadotropin releasing hormone (GnRH) and neuropeptide Y (NPY). We have been examining modulatory effects of GnRH and NPY on the olfactory epithelium of aquatic salamanders, axolotls (*Ambystoma mexicanum*). Although GnRH is often associated with reproduction and NPY with feeding, our studies indicate that the modulatory effects of both peptides vary in complex ways: their effects depend on the animal's sex, nutritional state, and reproductive state, as well as the behavioral significance of the odorant. For example, GnRH modulation of electro-olfactogram (EOG) responses evoked in adult females by whole-body odorants from other females varies with reproductive state (quantified by gonadosomatic index, or GSI) and nutritional condition: responses increase in magnitude in low-GSI, food-deprived females; remain constant in low-GSI, well-fed females; and decrease in high-GSI females. In contrast, we find that isoamyl acetate evokes fairly constant responses in axolotls exposed to GnRH, NPY, or a control treatment (Ringer's solution), suggesting that responses to this behaviorally meaningless odorant are not modulated. Finally, we find that the secretory potential that follows the neural component of the EOG response is also modulated by GnRH and NPY, but that changes in secretory responses are not correlated with changes in neural responses. Our results suggest that the brain regulates activity in the olfactory epithelium to emphasize stimuli that are most relevant to the animal's current behavioral and physiological state. Our results also indicate that the nature and consequences of neuromodulation in the olfactory system cannot be fully understood by simply studying laboratory animals that are well fed and in good reproductive condition.

Poster session II Poster #200

Perceptual responses to odorant mixtures originate mainly from peripheral processing

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Most natural odors are perceived from mixtures of odorants. Numerous studies reported that the perceptual characteristics of odorant mixtures are often different from those of their individual compounds. It has been shown that mixtures' intensity can be higher or lower than the simple arithmetic sum of each component's intensity. Besides, mixtures of odorants can also give rise to novel odor qualities (configural perception). The mechanisms that govern odor mixtures perception remain unclear, but there is increasing evidence that peripheral processes could play an important role in mixture processing. Indeed, competitive and non-competitive interactions have been observed at the olfactory sensory neuron level. In addition, using *in vitro* approaches, it has been shown that some odorants can act both as agonists or antagonists of olfactory receptors.

In the present study, we investigated in parallel the responses of olfactory receptors and behavioural responses to a binary mixture of odorants, octanal and citronellal, which has been shown to produce a configural (synthetic) perception in rats (Kay et al., 2003). Two experiments were conducted: (i) calcium imaging of HEK293T cells (Human Embryo Kidney cells) heterologously expressing human olfactory receptors: (ii) psychophysical measurements of the odor intensity and quality for the mixture and its components. In the *in vitro* study, the citronellal - octanal mixture was found to induce different effects (subtraction, compromise, partial addition), depending on the proportions of compounds in the mixture. In the human study, we observed that the addition of increasing concentration of octanal decreased the perceived intensity of citronellal in the mixture. Taken together, these findings support the hypothesis that interactions occurring at the olfactory receptor level contribute significantly to the olfactory coding of odorant mixtures.

Poster session II Poster #244

Altered olfactory performance in disinhibited states

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Human olfaction is characterized by high acuity yet low awareness to odors. This state has merited the hypothesis that the human olfactory system is under significant cortical inhibition. We hypothesized that reducing cortical inhibition would improve olfactory performance. To test this, we measured olfactory performance in disinhibited states. In three separate experiments, we used hypnosis, transcranial direct current stimulation (tDCS) and alcohol ingestion for reducing inhibition. In the hypnosis experiment we used an ascending-staircase three-alternative forced choice (3AFC) detection threshold test. In the tDCS experiment we tested olfactory identification (UPSIT) and 3AFC discrimination. In the alcohol-ingestion we used all three above tests.In the hypnosis experiment, of the 11 subjects we tested (5F, age 26±0.5), 9 had better olfactory performance under hypnosis versus wake (binomial, $p < 0.04$). This reflected a median 10% ± 9 improvement in the mean of the last 4 dilution steps (14% ± 5 in the 9 that improved).In the tDCS experiment, in the 34 subjects we tested (18F, age 26.3±0.8), tDCS did not influence3AFC discrimination (before tDCS: 0.48±0.04%, after: 0.43±0.03%, ($t(33)=1.2$,$p=0.2$), before sham: 0.53±0.04%, after: 0.50±0.03%, ($t(33)=0.7$, $p=0.5$)), nor did tDCS influence identification (UPSIT performance ratio, *after* divided by *before* the intervention (Active or Sham):sham:1.06±0.09, tDCS: 1.02±0.05, ($t(33)=0.34$, $p=0.73$).In the Alcohol experiment, we tested 6 subjects so far (3F, age 28.2±0.9). Although this sample is yet sufficient for statistical analysis, so far whereas detection threshold was poorer after alcohol in 4 of 6 subjects, discrimination and identification were unaffected by alcohol consumption. Although the current results do not support our hypothesis, the improvement in olfactory performance to a point better than baseline following hypnosis suggests powerful top-down influences in olfaction by mechanisms that remain to be uncovered.

Poster session II Poster #170

Bcl11b/Ctip2 controls the differentiation of vomeronasal sensory neurons in mice

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The transcription factor Bcl11b/Ctip2 plays critical roles in the development of several systems and organs, including the immune system, CNS, skin, and teeth. Here, we show that Bcl11b/Ctip2 is highly expressed in the developing vomeronasal system in mice and is required for its proper development. Bcl11b/Ctip2 is expressed in postmitotic vomeronasal sensory neurons (VSNs) in the vomeronasal epithelium (VNE) as well as projection neurons and GABAergic interneurons in the accessory olfactory bulb (AOB). In the absence of Bcl11b, these neurons are born in the correct number, but VSNs selectively die by apoptosis. The critical role of Bcl11b in vomeronasal system development is demonstrated by the abnormal phenotypes of Bcl11b-deficient mice: disorganization of layer formation of the AOB, impaired axonal projections of VSNs, a significant reduction in the expression of vomeronasal receptor genes, and defective mature differentiation of VSNs. VSNs can be classified into two major types of neurons, vomeronasal 1 receptor (V1r)/Gi2-positive and vomeronasal 2 receptor (V2r)/Go-positive VSNs. We found that all Gi2-positive cells co-expressed Go during embryogenesis. This co-expression is also observed in newly differentiated neurons in the adult VNE. Interestingly, loss of Bcl11b function resulted in an increased number of V1r/Gi2-type VSNs and a decreased number of V2r/Go-type VSNs, suggesting that Bcl11b regulates the fate choice between these two VSN types. These results indicate that Bcl11b/Ctip2 is an essential regulator of the differentiation and dichotomy of VSNs.

Poster session II Poster #362

Ultrastructural properties of parabrachio-thalamic and cortico-thalamic synapses in gustatory thalamus.

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Ventroposterior parvocellular nucleus (VPpc) is the thalamic relay of gustatory sensation to insular cortex (IC), receiving its primary input from the oral cavity via nucleus tractus solitarii and parabrachial nucleus (PBN). While explorations in sensory nuclei revealed principles of information processing in other sensory thalamic nuclei, our undertstanding of the gustatory thalamus have been rudimentary. We examined EM morphology of synaptic circuitry in VPpc originating from the PBN (parabrachio-thalamic; PT) and the IC (cortico-thalamic; CT). Anterograde tracer injected into the waist region of PBN led to prominent fiber labeling in VPpc, within a 400um X 500mm thin strip, located dorso-medial to medial lemniscus. At the electron microscope resolution, PT axons are myelinated, and they bear exceptionally large terminal boutons; PT terminals were more than 5X larger than unlabeled terminals, while they constituted about less than 3% of all synapses. Boutons contain dense core vesicles, resembling larger-sized CGRP terminals within VPpc. Terminal boutons form asymmetric, adherent and perforated synapses onto large-caliber dendrites and dendritic appendages from non-GABAergic neurons. PT boutons are often encased in glia; however glomeruli and triadic arrangements, characteristic features of other sensory thalamic nuclei, are not encountered. 3D reconstructions revealed that PT terminals formed multiple synapses at proximal dendrites as they emerge from soma. Tracer injections into IC led to filling of a dense network of fine fibers bearing small boutons in VPpc. CT terminals frequently synapsed on relay cells retrogradely filled from the same injections. EM morphology and targeting properties of CT axons are distinctly different than the primary PT inputs. Our results reveal unique properties of rodent thalamic gustatory processing, and they also allow differential comparison of the anatomical basis of functional processing among thalamic sensory nuclei.

Poster session I Poster #19

Dopamine role in behavioral and peripheral responses to plant volatiles in a moth

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Mating induces a switch in the plant attraction behavior in females of our model species, the Egyptian cotton leaf worm moth, Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) (Saveer et al[1]). In the wind-tunnel, virgin females are attracted to floral volatiles, whereas mated females are attracted to green leaf volatiles (GLVs). The mating status also modulates the tuning of the peripheral olfactory system: electro-antennography responses to flower volatiles and GLVs change according to the mating status in females.

Neuromodulators, such as biogenic amines can modulate the insect behavioral response by acting directly or indirectly on the peripheral or central nervous levels. Through High Performance Liquid Chromatography-Electrochemical Detection analysis, dopamine levels, but not the levels of other biogenic amines, were observed to significantly increase after mating in the primary olfactory centers, the antennal lobes, of female moths. Dopamine level is thus tentatively linked to the mating status, indicating that this dopamine could mediate directly or indirectly the plant behavioral switch of the females. Systemic injections of L-DOPA in virgin females were able to switch the behavioral and peripheral responses in virgin females to that observed in mated females. In addition, systemic depletion of dopamine in mated females, with α-methyl-tyrosine injections, restored virgin behavioral and peripheral responses in mated females. We conclude that dopamine either directly or indirectly modulates the plant attraction behavior of S. littoralis females and that this neuromodulation takes place partly in the peripheral olfactory system of this moth.

[1]Saveer A et al. Floral to green - mating switches moth olfactory coding and preference , In Press

Poster session I Poster #245

Energy, melancholy, desire… Are my olfactory feelings the same as yours?

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Gender, age and culture are among the most significant factors of inter-individual variations in olfactory perception. Women generally outperform men in cognitive treatment of odors; experience enhancement and cognitive development/impairment modulate odor perception; and odor-related practices/beliefs influence olfactory percepts too. The effects of these factors have been extensively studied for affective responses to odors such as pleasantness ratings. We used a more comprehensive approach, based on self-report Emotion and Odor Scales, which comprise about thirty terms organized in 6 to 7 dimensions. These scales have been developed in several cultures of Europe (British and Swiss), Asia (Chinese and Singaporean) and America (Brazilian and two North American). To construct these scales, a large initial pool of affective terms was reduced through a 3-step procedure to a smaller set of the most relevant terms, based on the ratings of 350 to 550 adult participants belonging to each culture. Participants had to rate the capacity of the terms to appropriately describe affective feelings experienced while smelling various odorants. Structural equation modeling approach showed that the structure and content of the scales comprise components shared across cultures but also culture-specific parts. The level of implicit and explicit knowledge about odors (familiarity / identification) was linked to intensity of affective responses to odors (pleasantness ratings / scores to the Emotion and Odor Scales). Age and gender were significant factors of variation for these different olfactory variables. The results can be related to more general emotional functioning and olfactory abilities, and to the main functions of olfaction in people's everyday life.

Contributed talks III "Mixed session" Monday 25 June

Multisensory perception of dietary faty-acids in *Drosophila*

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Fatty-acids (FAs) are crucial for animal survival and reproduction. However, our knowledge of the mechanisms underlying the perception and preference of dietary FA is limited, particularly in invertebrates. We obtained behavioral data with wild-type *Drosophila melanogaster* larvae and adults showing a clear preference to some of the FAs tested (C14:0, C16:0, C18:0, C18:1, C18:2, C18:3). These data, based on tests involving both individuals and groups, showed that larvae prefer desaturated FAs whereas adults prefer saturated FAs. Moreover, we found that larval and adult responses relied on olfaction and taste modalities, and maybe also on mechanoperception [1].

We will present our recent data obtained with *desat1* mutants (desat1 is involved in lipid metabolism), and with lines raised on FA-deprived food. In both cases, larvae show a partially altered response to specific FAs. This indicates that different sensory modalities underlying the perception of FAs, can be separately altered either by a genetic mutation or by a peculiar diet. We have also experimentally selected a line where larvae showed a reduced response to C18:0, which is normally strongly aversive. The larvae of these different lines varied for their lipid profiles. Together, these experiments suggest the existence of a possible link between perception and metabolism of FAs in *Drosophila*.

[1] Fougeron , AS., Farine, JP., Flaven-Pouchon, J., Everaerts, C., and Ferveur, JF. (2011). Fatty-acid preference changes during development in *Drosophila melanogaster*. *PloS ONE* **6(10):** e26899.

Symposium 10 "From odorant receptor to glomerulus" Sunday 24 June

Pharmacology of odor receptors

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Odor receptors are the largest family of GPCR's on the planet. They have much in common with the other 450 or so GPCRs found in the mammalian genome and can be profitably studied by using techniques for GPCR pharmacology – medicinal chemistry, structure activity relations, and a variety of modeling techniques. We have utilized synthetic chemistry to examine the structure function activity of an odor receptor by utilizing a variety of ligands that demonstrate a mechanisms for agonism, antagonism, partial agonism and reverse agonism. These analyses provide a theoretical picture of the requirements for a binding region within the receptor. The existence of a range of ligands – from antagonists to high affinity agonists for a given receptor - must be taken into account when determining the likely mechanism(s) used by upstream brain circuits to treat olfactory sensory input.

Poster session I Poster #155

Chemo- and thermosensory signaling in the Grueneberg ganglion

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The Grueneberg ganglion (GG) - a cluster of neurons in the anterior nasal region - projects axonal processes to the olfactory bulb and expresses the olfactory marker protein (OMP) as well as distinct olfactory receptors, suggesting a potential olfactory function of the GG. Searching for odorous cues stimulating the GG, we have recently identified a limited set of defined odorants – in particular dimethyl pyrazines - which activate murine GG neurons. Responsiveness to these odorants was confined to a larger subset of GG neurons which is characterized by the expression of the olfactory receptor V2r83, the transmembrane guanylyl cyclase subtype GC-G and the cyclic nucleotide-gated ion channel CNGA3. Experiments with knockout animals disclosed that GC-G and CNGA3 are important for odor-evoked GG responses.

In addition to odorants, GG neurons were also found to be activated by another environmental stimulus: cool ambient temperatures. Attempts to unravel the relevant signaling mechanisms revealed that almost all V2r83-/GC-G-/CNGA3-positive GG neurons responded to coolness, i.e, the same subset of GG neurons is activated by coolness and the above mentioned odorants. Experiments with GC-G- and CNGA3-deficient mice demonstrated that these elements contribute to coolness-evoked responses in the GG. Searching for a potential thermosensor, expression of the thermosensitive ion channel TREK-1 was observed in numerous GG neurons.

Sharing common transduction elements such as GC-G and CNGA3, attempts were made to evaluate whether cross-talks between the coolness-induced and the odorant-activated signaling pathway exist in GG neurons. The results indicate that temperature stimuli markedly affect odor-evoked responses in the GG.

This work was supported by the Deutsche Forschungsgemeinschaft.

Poster session II Poster #320

Taste receptors as nutrient sensors in the heart

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There is a growing appreciation that chemosensory (mainly taste and odorant) GPCRs are expressed in tissues beyond the oronasal cavity, where they have been ascribed novel functions in the brain, gastrointestinal tract and airway smooth muscle. In the heart, GPCRs are critical to physiological function and have proven to be rich therapeutic targets for cardiovascular disease. Given that several lines of evidence show that individual heart cells express >100 different GPCRs – not including the large families of chemosensory receptors which are yet to be systematically studied in the heart – it is possible that important physiological targets remain to be identified. Here, we performed a screen of taste receptor transcripts in rat heart and showed that seven Tas2r bitter taste receptors, as well as Tas1r1 and Tas1r3 (comprising the umami receptor) are expressed in neonatal whole hearts and in isolated, purified cardiomyocytes and cardiac fibroblasts. In addition, *in vivo* Tas1r1-r3 expression levels remained constant in the heart from birth to old age, while the Tas2rs displayed dramatically different temporal patterns of expression (Tas2r120 and Tas2r121 increased in adult hearts relative to neonates, while the remaining Tas2rs were expressed at lower levels in adult hearts). We have validated the expression of cardiac-expressed taste receptors using *in situ* hybridization and have confirmed the expression of Tas1r1 using gene-targeted mice ($Tas1r1^{Cre}$/$Rosa26^{tdRFP}$). Intriguingly, several of the taste receptors are upregulated in the heart in a model of starvation and we are currently exploring the potential function for taste receptors as cardiac nutrient sensors.

Poster session I Poster #201

Membrane potential slow oscillations of mitral/tufted cells: from shapes to mechanisms

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Mitral/Tufted cells, the output neurons of olfactory bulb, exhibit slow oscillations of the membrane potential coupled to respiration. In this study, we recorded intracellular activity of mitral/tufted (M/T) cells to characterize the shape of membrane potential slow oscillations (MPSO) and to uncover their origins.

We showed the existence of different shapes: positive MPSO (asymmetric with a depolarization peak), negative MPSO (with an hyperpolarisation peak) and symmetric MPSO. Interestingly, the occurrence, amplitude and phasing with respiration of these different MPSO shapes were modulated by odor stimulation and excitability changes. In particular, odor stimulation increased the occurrence and amplitude of MPSO, mainly of the negative type. In contrast, the percentage of positive MPSO was increased by hyperpolarization of membrane potential and the oscillation peak occurred earlier in the respiratory cycle for larger hyperpolarisation.

Surprisingly, some celles with respiration-synchronized discharge pattern did not exhibit any observable MPSO at resting membrane potential. However, we showed that these cells received a rhythmic input too. This was demonstrated by revealing an MPSO by DC current injection. On the contrary, we did not reveal any MPSO for cells without respiration-synchronized discharge. Using an M/T cell model, we proposed a mechanism for such "silent oscillations". We showed that a noisy synaptic conductance oscillation, whose reversal potential is close to the cell resting membrane potential, could lead to a rhythmic cell discharge without any MPSO.

Taken together, these results suggest that MPSOs participate to the synchronization of discharge activity on the

respiratory cycle. In addition, we postulate that positive MPSOs are a mixture of excitatory and inhibitory synaptic inputs while negative MPSOs mainly have an inhibitory source.

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Poster session II Poster #186

A phylogenetic analysis of vomeronasal receptors, V2Rs, in rodent species

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Two different neuronal populations exist in the basal VNO of mouse and rat. One population expresses phylogenetically ancient V2R families that are found in other animal species, including terrestrial and marine vertebrates. The other population expresses multiple combinations of V2R subfamilies and class Ib MHC molecules that were more recently established in an as yet unknown murine ancestor (Silvotti et al., 2011, PLoS ONE, 6, e24462). This complex organisation of the vomeronasal organ that exclusively developed in some rodent species could provide a molecular rationale for their exquisite chemosensory ability in individual recognition and mate choice, a prominent feature of these species.

In this work, we have carried out a phylogenetic analysis of vomeronasal receptors, V2Rs, using molecular and bioinformatics tools, in order to identify the position in the phylogenetic tree of rodents (Rodentia order) at which V2R expansion has occurred with the resulting establishment of a new population of vomeronasal neurons. We have also analysed if V2R expansion is associated with the appearance, in the rodent genome, of class Ib MHC genes and genes encoding specific protein pheromones.

Poster session II Poster #20

Left-right asymmetry of olfaction in *Apoidea species*

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We investigated the olfactory learning and responses of the right and the left antenna in three species of Hymenoptera Apoidea: eusocial honeybees (*Apis mellifera* L.), mason bees (*Osmia cornuta* L.), a solitary species, and bumble bees (*Bombus terrestris* L.), an annual eusocial species. By training bees on the proboscis extension reflex paradigm (PER) with only one antenna in use, we found asymmetrical performance favouring the right antenna in responding to learned odours in honeybees and bumble bees, but not in mason bees. Honeybees appear to be lateralized at the population level (more than 50% of the individuals showing a similar direction of bias) in both behavioural (conditioning of the PER) and physiological (ElectroAntennoGraphy, EAG) responses (with a dominance of right-sides structures), whereas, mason bees appear to be lateralized only at the individual level. In the honeybees, lateralization for short-term memory recall of PER seems to be correlated with a difference in the number of olfactory sensilla, which is significantly higher on the right than on the left antenna. In bumble bees electroantennographic responses did not reveal, however, significant antennal asymmetries in odour detection, whereas morphological counting of olfactory sensilla showed a predominance in only one type of receptors, with a higher number of olfactory sensilla trichodea type A in the right antenna. The occurrence of a population level asymmetry in olfactory learning of bees provides new information on the relationship between social behaviour and the evolution of population-level asymmetries in animals. Overall, results seem to support the hypothesis that brain and behavioural lateralization at the population level have evolved under social selective pressures as a strategy to optimize coordination among asymmetrical individuals.

Poster session I Poster #263

The effect of labels on odor perception

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We react faster to unpleasant odors compared to pleasant odors; this is especially true for food odors. Further, when an odor is labelled positively, subjects evaluate its intensity, pleasantness and edibility differently than when the same odor is labelled negatively. In this project we aim to investigate the effect of labels on odor perception more closely. Specifically, we tested whether negative labels of five different odorants presented birhinally led to shorter response times than positive ones. We also examined how the subjects evaluated the edibility, pleasantness and intensity of odorants as a function of their label.

Preliminary results from 17 participants did not show yet a significant effect of odor label on response times. However we found a significant effect of odor on response times; participants seem to react faster to some odors rather than others. Further, all odors were significantly rated as being more pleasant and more edible when labelled positively as compared to the negative labels.

These preliminary results seem to confirm the reported effects of labels on odor perception.

Poster session I Poster #279

Crossmodal integration of congruent olfactory-visual stimulus combinations is valence dependent

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Rarely, an odor is perceived in isolation, but input from other senses concordantly occurs. We recently demonstrated that visual stimuli increase perceived pleasantness of a congruent odor, while olfactory sensitivity and perceived olfactory intensity were not affected by visual stimulation. However, to date, only pleasant olfactory-visual combinations have been tested. The aim of the current study was to explore influence of visual congruency on olfactory sensitivity, intensity, and pleasantness evaluation using both pleasant and unpleasant odors. To this end, olfactory detection thresholds for the generally pleasant odor phenyl ethyl alcohol (PEA) and generally unpleasant odor isovaleric acid (IVA) were tested in combination with a congruent, incongruent, or blank visual stimulus in 38 healthy, normosmic participants. Additionally, we obtained intensity and pleasantness ratings of the pleasant odors PEA, strawberry, and peach, as well as the unpleasant odors IVA, fish, and manure. We were able to show that ratings of pleasantness and intensity of pleasant odors are influenced by visual congruency. Congruent pleasant odor-picture pairs are rated as more pleasant and more intense than incongruent pairs. This effect is not odorant-specific and did not occur for unpleasant odors. Moreover, as previously demonstrated, olfactory sensitivity is not influenced by visual input, independent of pleasantness of the stimuli. The absence of influence of visual stimuli on unpleasant odors implies its evolutionary importance as warning cues; i.e. the unpleasant odor itself represents a salient signal, which is not amplified by a congruent visual stimulus, thus indicating superiority of processing of negative odors. In conclusion, odor-visual congruency processing is influenced by the underlying perceived valence of the stimuli.

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Poster session II Poster #316

Olfactory function after transnasal hypophysectomy

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Introduction: Transnasal surgery of the pituary gland is potentially harmful to the olfactory epithelium. Several authors have already investigated olfactory function before and after transnasal hypophysectomy. So far, retronasal olfactory function has never been studied after this surgery. Since the surgical field is close to the sphenoid sinus it is hypothesized that retronasal olfactory fucntion could be more concerned than orthonasal olfactory function

Aim of the study: To investigate ortho- and retronasal olfactory function in patients undergoing pituary gland surgery.

Material and Methods: We included 56 patients and tested ortho- and retronasal olfactory function psychophysically before and 3 month after surgery.

Results: No patient lost olfactory function after surgery. Eight percent of the patients complained of altered olfactory function after surgery. However, no major change in olfactory function could be found three months after surgery. Neither ortho- nor retronasal olfactory function were significantly modified after surgery.

Conclusion: These results do not confirm previous reports about a large numbers of patients with olfactory disorders due to surgery. They further suggest that this is a safe surgery regarding preservation of olfactory function.

Symposium 14 "Higher olfactory processing - Delwart Symposium" Tuesday 26 June

Neuronal filtering of synthetic odor representations in a cortex-like brain area

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We analyze neuronal computations in the olfactory bulb and cortex by a combination of optical, physiological, molecular and theoretical approaches in a small vertebrate model system, the zebrafish. I will focus on three recent findings. First, we found that odor representations across olfactory bulb output neurons are largely invariant to changes in odor concentration but switch abruptly when one odor is morphed into another. The olfactory bulb therefore classifies sensory inputs into a large number of discrete outputs. This computation creates defined, noise-limited stimulus representations and acts as a sensory filter. Second, we found that telencephalic area Dp, the main target of the olfactory bulb in zebrafish and the homolog of olfactory cortex, uses multiple synaptic pathways to integrate sensory information across processing channels in the olfactory bulb. This integration is thought to establish synthetic representations of olfactory objects. Third, using optogenetic manipulations of activity patterns in the olfactory bulb and odor stimulation, we found that neuronal circuits in area Dp perform at least two temporal filtering operations that tune Dp neurons to those features of input activity patterns that are particulary informative about precise odor identity. These temporal filtering mechanisms differ from those in insects, possibly because precise odor information is represented differently in the olfactory bulb and in the antennal lobe. Together, these results provide insights into the transformations of odor representations in the olfactory bulb and its cortical target Dp.

Poster session II Poster #428

A method for purifying and identifying human chemosignals

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There is ample evidence for human chemosignaling. For example, components in human sweat can drive menstrual synchrony (the McClintock effect) or convey fear, and components in human tears mediate arousal. Only one sweat-bound component, however, has been specifically isolated and identified as a putative chemosignal. Androstedienone is a sex hormone-derived steroid more prevalent in human male than female sweat, and it induces a host of emotional, physiological, and endocrine responses primarily in women. The sweat-bound and tear-bound components responsible for the majority of the above-described effects remain unidentified. Here we describe the application of modern tools from analytical chemistry towards the goal of identifying the specific compounds responsible for these effects. We describe a method for first collecting samples directly from the human body under different conditions, for example, emotional tears versus reflex tears, or from different genders. We then use an electronic nose to ask whether the different samples form clusters in chemical space. Testing 10 subjects (4F), we found that the two genders generate significantly separable reflex tears (Kruskal-Wallis $H=15.26$, $p<.0005$). We then use a use a Gas-Chromatograph Mass-Spectrometer equipped with a splitting line that allows smelling of individual components from each mixture. We deliver the components separately, concomitantly measuring physiological responses (GSR, Respiration, Heart Rate) and subjective behavioral parameters (Mood, arousal). We use a preparative fraction collector to enrich putatively active signaling molecules, which we can then deliver in an fMRI experiment, as well as submit to NMR analysis of structure for unknown compounds. In this poster we will display our current optimization scheme for this method, and point to initial candidate human chemosignals. Such compounds may have significant pharmacological implications.

Poster session I Poster #363

The effect of congenital loss of vision or smell on taste perception

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Introduction: Our sense of taste is highly multimodal and is strongly influenced by vision and smell. For example, water colored in red increases taste thresholds, even when subjects are firmly instructed to ignore the color (Verhagen and Engelen, 2006). Flavors are created by the combination of smell and taste as everyone has already undoubtedly experienced during a cold or flu. Thus, multiple senses contribute to the pleasure of eating and in judging the palatability of food. In this study, we address the effects of the absence of vision or smell from birth on the sensory and hedonic aspects of taste processing (Sewards, 2004).

Methods: A group of 10 congenitally blind, 17 congenitally anosmic/ hyposmic and 18 normal control subjects, matched for age and sex, participated in the study. Taste detection and identification thresholds of the five basic tastants (sweet, salty, acid, bitter and umami) were measured using the 2-cups "sip and spit" method (Hong et al., 2005). Sensitivity for phenylthiocarbamide bitterness was also assessed. Finally, participants completed several eating habits questionnaires (Neophobia Scale, Pliner and Hobden, 1992; Variety Seeking Tendency Scale, Van Trijp and Steenkamp, 1992 and Intuitive Eating Scale, Tylka, 2006).

Results: Psychophysical thresholds for taste detection and identification were not statistically different between the 3 groups. Results of the eating habits questionnaires indicate that the anosmic/hyposmic and blind subjects eat more intuitively when they feel hungry and do not use food intake to cope with negative affect like anxiety or sadness.

Conclusion: Absence of vision or smell from birth is associated with changes in the "hedonic" (as indicated by the results on the intuitive eating scale) but not in the "sensory" (as indicated by our psychophysical results) taste pathway. These data moreover suggest that subjects with congenital absence of vision or smell may be less prone to develop eating disorders.

Poster session I Poster #291

Impacts of a non-consciously perceived food odour on subsequent food-related behaviour

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Studies in cognitive psychology have highlighted a link between perception and action, by revealing the non-conscious influence that an olfactory cue can have on thinking and doing. A striking example is Holland et al.'s study (Holland et al. 2005). When incidentally exposed to the odour of a citrus-scented cleaner and while they had not noticed this odour, participants showed shorter reaction times concerning cleaning-related words in a Lexical Decision Task, and when asked to eat a biscuit, they removed the biscuit crumbs more often than participants non-exposed to the citrus odour. The present study aimed at exploring if the incidental exposition to an olfactory healthy food cue could have an impact on food choice. Fifty-eight participants took part in our study, and were assigned randomly to either a control or scent condition. In the scent condition, they were unobtrusively exposed to a melon odour, chosen as prototypical of the fruit category, in the waiting room, while in the control condition this room was non-odorized. They all then performed a Lexical Decision Task, and a choice on a menu task. Results showed first that participants from the scent condition answered faster, only for the word 'melon' in comparison to other tested words, suggesting the activation of a related concept in the brain. Second, participants from the scent condition showed proportion of healthy choices higher, only for starters in contrast to the other courses of the menu. These results support the idea of priming effects specific to a food cue, which have already been obtained in studies exploring olfactory priming in the food domain (Fedoroff et al., 2003; Coelho et al., 2009). Our study provides scientific evidence that a not consciously perceived food odorant, susceptible to be associated with a healthy food, can to some extent influence our food choices to healthier ones. Further research on these implicit processes will certainly enrich sensory and consumer research.

Symposium 18 "Olfactory neuroethology" Tuesday 26 June

Olfactory processing and behavior in honeybees

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All insects have a basically similar olfactory system, with a glomerular antennal lobe being the first neuropil that processes olfactory information. Nevertheless, when looking at the detail, there are important differences. For example, the honeybee olfactory system consists of 60.000 receptor cells on each antenna, 160 glomeruli in each antennal lobe, about 3000 local neurons in the antennal lobe, and 800 projection neurons which leave the antennal lobe towards the mushroom bodies using two distinct tracts, the mAPT and the lAPT. Other insects have different numbers. Most importantly, however, numbers do not just scale, but appear to be qualitatively different. Thus, the number of local neurons in bees is disproportionally larger than in flies and moths. Also, local neuron morphology and transmitters differ. Furthermore, the two distinct tracts of projection neurons vary widely across insect species with respect to their prominence. In this talk, I will present data about the architecture and function of the honeybee olfactory system, including an analysis of local neuron types.

Symposium 8 "Central mechanisms of taste learning and memory" Sunday 24 June

Temporal lobe circuits involvement in taste recognition memory

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Taste neophobia is used as a novelty index in rats. When the ingestion of a novel taste solution is not followed by negative consequences it becomes recognized as safe. Thus, safe taste recognition memory leads to increased consumption of familiar tastes, a non-associative learning process named habituation of neophobia. The existence of shared neural circuits and molecular mechanisms in taste memory and other types of recognition memory, such as object recognition memory is controversial. This controversy has important theoretical implications for the current memory classifications, since recognition memory is considered as a type of declarative memory while habituation of neophobia is classified as non-declarative memory relying on distinct neural circuits.We have applied an approach which combines lesion and inmunohistochemical identification of immediate early genes as neuronal activity markers in order to delineate interdependent components of the neural circuit involved in safe taste recognition memory. The results have shown increased activity of the medial portion of the perirhinal cortex but not the dorsal hippocampus induced by exposure to familiar taste solutions. This increased perirhinal activity depends on the basolateral amygdala integrity, which is consistent with previous data obtained in lesion studies. In addition, neurotoxic lesions of the basolateral amygdala disrupt changes in the activity of thalamic taste relay nuclei induced by taste familiarity. Therefore, our results point to the perirhinal cortex as a component of the neural circuit required for safe taste memory in rats. This is in accordance with an involvement of the area in other types of recognition memory, such as visual recognition memory. However, anatomical dissociation among shared and independent components of the temporal lobe and subcortical areas required for various types of recognition memory are also evident.

Supported by HUM 02763, PSIC2008-03933 and PSIC2011-23702.

Poster session I Poster #21

Diacylglycerol modulates pheromone-dependent signal transduction in the olfactory receptor neurons of the hawkmoth *Manduca sexta* in a time-dependent manner

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Odor transduction in insects is still under debate. In moth antennae pheromones activate phospholipase Cβ and cause equimolar rises in IP3 and diacylglycerol (DAG) while in the fruitfly an odor-gated ionotropic mechanism could be shown. So far the role of DAG in pheromone transduction is not completely understood. With extracellular tip recordings from pheromone-sensitive trichoid sensilla in the hawkmoth Manduca sexta we investigated whether directly DAG-gated TRP-like ion channels or the activation of protein kinase C (PKC) are involved in pheromone detection. The DAG-analogues, DOG and OAG, as well as the PKC inhibitor Gö6976 were passively perfused into the sensillum lymph. Pheromone responses to brief pulses of bombykal (1µg BAL), the main pheromone component of female hawkmoths were recorded. The experiments were performed at the end of the activity phase and the resting phase to search for Zeitgebertime (ZT)-dependent differences. In contrast to DOG, application of OAG increased the BAL-dependent sensillum potential amplitude at both ZTs. Additionally, while OAG elevated the frequency of the first 5 action potentials of the response maximally during the moth´s activity phase, DOG reduced it. Infusion of Gö6976 decreased both parameters of the BAL response with a significantly stronger effect during rest. Thus, we suggest that DAG plays an important role in the pheromone-dependent signal transduction of M. sexta, apparently depending on the circadian rest-activity cycle of the moth. Furthermore, we assume that OAG mostly targets TRP-like ion channels while DOG activates PKC. Future experiments will challenge our hypothesis that weak BAL stimuli cause DAG-dependent activation of transient Ca2+ channels while adapting pheromone stimulation leads to stronger Ca2+rises and DAG activates PKC to further allow pheromone responses under conditions which close DAG-dependent ion channels. [Supported via DFG grant STE 531/20-1 to MS and the Schwerpunktprogramm SPP 1392]

Contributed talks II "Gustation" Monday 25 June

Taste stimuli regulate peptide hormone secretion from gustatory epithelium

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The taste bud produces myriad peptide hormones including glucagon, glucagon-like peptide-1 (GLP-1), cholecystokinin and neuropeptide Y. These peptides and their cognate receptors show distinct expression patterns in the taste bud and can exert discrete effects on taste cell physiology. Furthermore, genetic and pharmacologic disruption of specific peptide signaling pathways indicates important contributions to the modulation of peripheral taste function. For example, mice exhibit reduced behavioral responsiveness to sweet stimuli upon disruption of either GLP-1 or glucagon signaling. However, the conditions under which these peptide hormones are secreted is unknown. We developed a modified Ussing chamber approach to measure the effects of taste stimuli on peptide hormone secretion from mouse circumvallate (CV) papillae. This approach allows isolation of the CV epithelium such that stimuli are restricted to the apical surface and secreted peptides can be collected from the basal side. We also assessed the contribution of the sweet and umami taste receptors by comparing hormone secretion from T1R3-/- mice and their heterozygous controls (T1R3+/-). Hormone levels were determined by ELISA. Both glucose (25-250 mM) and monosodium glutamate (MSG; 25-200 mM, with 1 mM IMP and 50 μM amiloride) increased GLP-1 secretion from T1R3+/- CV in a concentration-dependent manner ($p<0.0001$). However, GLP-1 secretion from CV of T1R3-/- mice remained at basal levels for all stimulus concentrations. In contrast, both glucose and MSG inhibited glucagon secretion in T1R3+/- CV ($p<0.0001$); neither stimulus reduced glucagon secretion from T1R3-/- tissue. Together, these data indicate that the secretion of both GLP-1 and glucagon from mouse taste cells is regulated by appetitive taste stimuli and by the taste receptor T1R3. Support: NIDCD (DC010110), Ajinomoto Amino Acid Research Program.

Poster session II Poster #22

Integrated dual signaling in the *Drosophila melanogaster* olfactory receptor complex increases sensitivity and speed of odor detection

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Insects are highly dependent on the sense of smell. Their olfactory system should therefore optimize both sensitivity as well as speed of odor reception and thereby signal transduction. We hypothesize that a combination of metabotropic and ionotropic signaling mechanisms is responsible for detection and amplification of brief and intermittent odor signals in insect olfactory sensory neurons (OSN) expressing the co-receptor protein Orco. To test our hypothesis, we used electrophysiological recording in wild type and transgenic *Drosophila melanogaster* flies. Different types of OSNs were challenged with odorant stimulation presented at different frequencies (1 Hz to 10 Hz), using 10 consecutive 50ms pulses. OSNs could typically follow pulses up to a frequency of 5 Hz. In transgenic flies carrying mutations in Orco phosphorylation sites (PKC), olfactory responses exhibited reduced magnitude and required longer stimulus times to respond to odor stimulation. These mutant OSNs also showed longer response latencies and altered temporal response dynamics at lower doses. However, PKC flies could respond and follow pulses of brief stimulation when the concentration was elevated to 100 times as compared to the wild type stimulations. We conclude that dual and synchronized signaling in OR-Orco-expressing OSNs optimizes both sensitivity and speed of odor detection and might improve pulse resolution of intermittent stimuli encountered in odor plumes.

This research was funded by the Max Planck Society

Poster session I Poster #429

Expression profiling of olfactory receptors by next generation sequencing

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Olfactory receptors (ORs) provide the molecular foundation for the detection of volatile odorant molecules from the environment. Recent studies showed that the expression of these receptors is not restricted to the olfactory epithelium (e.g. testes, prostate and gut). The aim of this work is to create a comprehensive expression analysis of murine olfactory epithelium and ectopically expressed olfactory receptors in several tissues of the human body. Isolated RNAs from human and murine tissues were analyzed by Next Generation Sequencing with the Illumina Genome Analyzer II. In addition, we reanalyzed data of already published sequencing projects for the expression of ORs.

Our analysis of murine olfactory epithelium (OE) transcriptome confirms the strong expression of genes participating in olfactory signal transduction. In murine OE, expression of > 1000 OR genes was detected whereas in non OE tissues, fewer ORs were found, for example ~100 in murine testis.

Characterization of human tissues revealed ectopical expression of a considerable number of ORs outside the OE. For several tissues, OR expression was verified by semi-quantitative RT-PCR. We could observe the presence of ORs which were expressed in one particular type of tissue as well as ORs which presented a broader distribution. The further characterization of these ORs will provide new insights to the physiological role of distinct ORs outside the olfactory epithelium.

Poster session I Poster #23

Chemosensory adaptation to host-plant in a noctuid

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Insects mainly use their chemical senses (olfaction and taste) to detect and select an adequate host for feeding/ovipositing. In a context of global changes and anthropization, dynamic and genetic changes in insect pest populations menacing agriculture may occur (shifts in distribution range, adaptations to new plants and apparitions of pest traits). Since the chemosensory system of insects has been shown to evoluate rapidly, depending on the ecological context, we questioned if it could contribute to such host plant adaptation, using as a model organism the noctuid moth Sesamia nonagrioides, an important crop pest in Europe. We took advantage of the occurrence of two populations in Kenya and France, with the same origin but each adapted to different host-plants. The Kenyan population feeds on wild plants whereas the population found in south west of France has adapted to cultivated habitats and feeds almost exclusively on corns. A comparison between these two populations gives us an opportunity to study the phenomenon of host change under the influence of anthropization.

Based on next generation sequencing technologies, our approach consisted, as a first step, in establishing the chemosensory transcriptome and identifying chemosensory genes in this species. We have identified several genes coding for olfactory receptors including the obligatory co-receptor, for odorant-binding proteins including pheromone-binding proteins, for ionotropic receptors and other actors of the chemosensory mechanisms in S. nonagrioides. The second step is in progress and consists in using RNAseq to highlight genes differentially expressed between the two populations.

Poster session I Poster #281

Sniffing out a visual threat

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In a threat-detection task, humans detect an angry (threatening) schematic face in an array of neutral distracter faces more quickly than a friendly (non-threatening) face. This well-established angry advantage effect was replicated and extended in a recent crossmodal visual-olfactory study using schematic human faces and human body odors: the body odor of unknown individuals (Strangers) – an established threatening olfactory stimulus – speeds a subject's detection of threatening faces, but not non-threatening faces, relative to exposure to the subject's own body odor (Self). In the present event-related potential (ERP) study, we sought to identify the cortical processes mediating this effect. Angry and neutral schematic faces were presented to twenty-one subjects in the presence of Strangers' body odor (ST), Self body odor (SE), or an odorless control, which were delivered intra-nasally by a computer-controlled olfactometer. Perceptual ratings of odor intensity, pleasantness, and familiarity were also collected. To eliminate the potential confounds of single-donor body odor stimuli, super-donor ST stimuli were created by combining the body odors of four different, unknown individuals. Stimulation yielded clear visual ERPs, including face-dependent local minima over the occipital/parietal cortex and a late positive complex comprised of several smaller deflections. Preliminary analyses suggest that exposure to human body odor, relative to odorless control, results in significant differences in the late (cognitive) complex but does not affect early (sensory) components of visual processing. This suggests that body odor can modulate the cognitive evaluation of visual stimuli. The effects of Strangers' and Self body odor exposure on visual processing, specifically on the processing of threatening faces, will be presented and discussed within the framework of the adaptive advantages conveyed by heightened sensitivity to threat-related stimuli.

Poster session II Poster #156

Electrophysiological investigation of intrinsic mitral cell properties in the mouse accessory olfactory bulb

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The accessory olfactory bulb (AOB) represents the first relay station of information processing in the rodent accessory olfactory system. AOB mitral/tufted cells, which are the main excitatory projection neurons in the AOB, receive synaptic input from the sensory neurons of the vomeronasal organ. In turn, mitral cells are modulated by different classes of inhibitory neurons, such as granule and periglomerular cells.

Despite their physiological significance, the intrinsic properties of mitral cells and their role in the social information coding and signal integration in the AOB are not fully understood. Here, we investigate the biophysical properties of AOB mitral cells. Using both voltage-clamp and current-clamp whole cell recordings from optically identified mitral cells in acute mouse AOB tissue slices, we analyzed mitral cell ion channel properties. Using a pharmacological approach, we show that AOB mitral cells functionally express multiple voltage-gated ion channels, including a variety of voltage-dependent calcium channels.

We envisage that the physiological properties of mitral cells are critical for the faithful transmission of primary sensory signals from vomeronasal neurons, modulation of these signals and their relay to higher brain centers. Our data will advance the general understanding of the functional role of mitral cells in olfactory information coding and processing in the mammalian accessory olfactory system.

Symposium 7 "Human olfaction" Sunday 24 June

Human olfaction: linking odor objects to their names

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How the olfactory system and language system interact to generate names of odor objects is poorly understood. It has long been recognized that humans have great difficulty naming and identifying odors that are highly familiar to them, and show improvement only when provided with relevant non-olfactory cues. In this presentation I will discuss recent psychophysical and imaging findings from healthy subjects and patients with progressive language dysfunction (primary progressive aphasia) that help illuminate the neuroscientific basis of odor naming and mis-naming. Convergent anatomical and functional MRI data indicate that the temporal pole serves as an olfactory gateway to object identification areas, providing associative access to knowledge-based representations for naming selection and retrieval. Complementary behavioral work indicates that odor identification precedes, and perhaps even mediates, odor valence evaluations. Together these results suggest a causal framework to explain how object features are extracted from odors to guide olfactory cognitive and evaluative processes.

Supported by grants to J.A.G. from the National Institute on Deafness and Other Communication Disorders (NIDCD)

Symposium 22 "Odor memory and perception: cells to circuits" Wednesday 27 June

Encode, integrate, repeat: mechanisms of olfactory perceptual processing in the human brain

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Humans and other animals often make perceptual decisions on the basis of noisy sensory information. Neuroscientific research in this area has historically centered on the visual and somatosensory systems, but recent studies in the olfactory domain have brought important new insights regarding how the nervous system contends with stimulus unpredictability, and how animals adjust their behavior to optimize perceptual decisions. This presentation will highlight recent work from our lab focusing on the systems-level involvement of the human olfactory system in predicting, integrating, and consolidating perceptual information about odor objects. Using a combination of olfactory psychophysical approaches, pattern-based functional imaging techniques, and computational modeling, I will highlight the unique but complementary roles that piriform cortex and orbitofrontal cortex play in optimizing olfactory perceptual discrimination. Findings discussed here will highlight potential mechanisms by which the olfactory system can extract meaningful information from noisy inputs.

Supported by grants to J.A.G. from the National Institute on Deafness and Other Communication Disorders (NIDCD)

Poster session II Poster #24

An in vivo-atlas of the *Drosophila* antennal lobe based on receptor neuron targeting

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One of the most important requirements for the analysis of *in vivo*-imaging dataset is the availability of a 3D-atlas of the neuropil of interest, e.g. the antennal lobe (AL) the first olfactory neuropil. The most commonly used atlases for the AL in

Drosophila, are the ones generated by LAISSUE et al. (1999) and COUTO et al. (2005). They are based on either plain morphology or immuno staining.

These *in vitro* generated atlases have one general flaw making it difficult to utilize them *in vivo*. As the brain is dissected out of the head capsule, the antennal nerve is cut. Without any attachment of the nerve, the AL is lacking the tension caused by it. This in turn results in a modified positioning of the subunits of the AL, the glomeruli, in relation to each other.

To solve this we generated a fly that expresses dsRed as a direct fusion with synaptobrevin and therefore enables staining of the neuropile in the living fly. Performing two photon microscopy we could reconstruct a 3D-model of the *in vivo* brain and subsequently identify glomeruli.

The identification was supported by additional labeling of specific receptor GAL4 lines, incorporating ORs as well as IRs, with GFP in the background of the synaptobrevin-dsRed. These functional identifications of glomeruli together with the morphological ones provide a functional atlas as the basis for an *in vivo* characterization of frequently used ubiquitous GAL4 lines. Utilizing the same background labeling we mapped the *in vivo* condition of the most commonly used lines in olfactory research not only regarding their plain glomerular repertoire but also their arrangement for simplified assignment of odor evoked imaging signals.

Further it is now possible performing ultrastructural analyses of the intraglomerular distributions of different neuronal populations *in vivo* leading to a more comprehensive morphological understanding of the combinatorial AL map.

Poster session I Poster #399

Can age-related CNS taste differences be detected as early as middle age? Evidence from fMRI

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Middle-aged Americans have the highest obesity rates of any age group in the United States, yet little is known about age-related changes in central taste function during this critical time. Research on taste and aging has primarily focused on psychophysical responses, and on older adults. Central taste processing in middle age has not been investigated. In the current study, we compared fMRI activation of young and middle-aged adults during hedonic evaluation of a sweet and a bitter taste. A 2 (age group) by 2 (tastant) analysis of variance (ANOVA) on fMRI activation revealed: (1) a main effect of age (young adults > middle-aged adults) in the bilateral anterior cingulate, lentiform nucleus, putamen, caudate head and body, and right precentral gyrus; (2) a main effect of taste (sweet > bitter) in the bilateral pre- and postcentral gyri, and anterior cingulate; and (3) an age by taste interaction in the bilateral pre- and postcentral gyrus, the left insula, and the left thalamus. We speculate that these results might reflect early age-related differences in central processing that occur prior to deficits in gustatory function observed in old age, and this might have important implications for weight changes that occur during middle age.

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Symposium 10 "From odorant receptor to glomerulus" Sunday 24 June

Determinants of mitral cell fate

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We recently demonstrated that the fate of olfactory bulb mitral cells is governed, in part, by the timing of neurogenesis. Both the location of the mitral cell in the olfactory bulb and its connections to cortical structures equated with a narrow window within the period of mitral cell genesis. Here, we have begun to explore further the molecular mechanisms underlying mitral cell fate and have turned out attention to transcription factors. Mitral cells are glutamatergic olfactory bulb projection neurons. Tracking mitral cell development with BrdU labeling and transcription factors characteristic of glutamatergic projection neurons, we found that Tbr1 expression in mitral cell precursors preceded Tbr2. These data suggest that mitral cells may bypass the stage of intermediate progenitor cell, which is observed during the differentiation of cortical pyramidal neurons. Using in utero electroporation, we also showed that down-regulation of Pax6 was necessary for the expression of Tbr1 and Tbr2 in mitral cell precursors. Therefore, sustained Pax6 expression in embryonic olfactory bulb neurons decreased the number of cells that progressed to a mitral cell fate. In contrast, sustained expression of Pax6 resulted in an increase of GABAergic and/or dopaminergic interneurons. These results indicate the existence of multi-potential progenitor cells in the nascent olfactory bulb, and that fate determination of precursor cells is cell-autonomously regulated by the levels of Pax6.

Poster session II Poster #150

The lack of Neurogranin (Ng) expression in Ng-KO mice induces morphological alterations in adult born olfactory granule cells without affecting their survival

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Neurogranin is a brain specific protein that controls the availability and distribution of calmodulin (CaM) at post-synaptic sites. The role of Ng in modulating Ca^{2+}/CaM dependent signal transduction has been correlated to synaptic plasticity in the adult hippocampus. We recently described Ng is expressed in the mouse olfactory bulb (OB), where it identifies a large population of mature GABAergic granule cells (GCs), preferentially distributed in the deep portion of the granule cell layer (Gribaudo et al., J Comp Neurol 2009). In the present study, we found that Ng-positive GCs highly co-express CaMKIV and CaMKII, both downstream proteins of the Ca^{2+}/CaM dependent pathway. Moreover, the Ng-phosphorylated form is enriched in OB synaptosomes, supporting an active role for Ng at synaptic sites in the OB. Birth-dating analysis shows that in adult generated GCs, Ng expression progressively increases in parallel with cell maturation, reaching a peak at 21 days post BrdU injection, in coincidence with an increase in spine density, to remain fairly constant at 42 and 63 days survival. Analysis of adult generated GCs in Neurogranin knock out (Ng-KO, Pak et al., 2000) mice indicates that the lack of Ng expression in these cells does not affect their survival but it induces morphological defects including: i) increase in the extension of the basal dendritic compartment; ii) modification in the ramification pattern of the apical dendrite proximal portion; and iii) reduction in the number of spines in both apical and basal dendritic compartments, supporting a role for Ng in the formation and stabilization of GC synaptic contacts. Wheter these morphological defects result in an alteration of GC excitability and/or olfactory-related behavioural deficits remain to be investigated.

Poster session I Poster #157

The distribution of α1- and α2-adrenoceptor subtypes in the accessory olfactory bulb of female mice

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The ability of mated female mice to recognise their mate's chemosignals is vital for their reproductive success, as it prevents the pregnancy block response to chemosignals from unfamiliar males. This mate recognition memory involves changes at the mitral/tufted (M/T) cell:granule cell reciprocal synapses in the accessory olfactory bulb (AOB), as a result of the mate's chemosensory input and coincident activation of the noradrenergic input from the locus coeruleus which is initiated by mating. The pharmacological manipulation of α, but not β, adrenoceptors has been shown to prevent mate recognition and previous studies have shown that individual subtypes of both α_1- and α_2-adrenoceptors can be found in the AOB. However, their detailed cellular distribution and the roles that they may play in modulating M/T cell activity in the AOB are unclear. We have used immunohistochemical staining to reveal distinct cellular localisations for α_{1A}-, α_{1D} -, α_{2A}- and α_{2C}-adrenoceptors in the AOB, with no significant staining for α_{1B} or α_{2B} receptors. The localisation of different adrenergic receptor subtypes, on both M/T cells and inhibitory interneurons, suggests a complex role for noradrenaline in modulating AOB function and inducing learning. We have also confirmed that artificial vaginocervical stimulation has a disinhibitory effect on M/T cell activity in anaesthetised mice. This preparation will be of use in future experiments using pharmacological or targeted genetic techniques to determine the role of the different adrenergic receptor subtypes in the induction of mate recognition memory.

Poster session I Poster #25

Experience-dependent plasticity of the peripheral olfactory code in *Drosophila melanogaster* larvae

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During their larval stage, fruit flies are exposed to an odour-rich environment, in which they must choose between toxic and edible substrates. For this they need an efficient olfactory system with the capacity for both short and long term plasticity based on experience. *Drosophila* larvae possess only 21 paired olfactory sensory neurons (OSN), most of which express only one olfactory receptor (OR) and the co-receptor Orco. Information arising from each OSN is transmitted to a unique glomerulus in the antennal lobe and then to the mushroom body via projection neurons. Combinatorial coding in the periphery allows larvae to detect and discriminate a large number of odours. Previous studies have characterised the odour-response profiles of 19 of the 21 OSNs and found that an OSN's response to a given odour is often highly variable (Hoare et al 2008, 2011). This raised the possibility that the peripheral olfactory code exhibits plasticity and that this plasticity may be directly involved in mediating behavioural adaptation and learning.

We are exploiting the UAS-Gal4 system to create single Or lines in which only one identified OSN is functioning. Using electrophysiology and behavioural assays, we are studying the effect of short-term adaptation on the response of individual OSN classes to a panel of odours. Preliminary data suggest that adaptation to some, but not all odours, is associated with changes in OSN responses supporting the hypothesis that short term plasticity of olfactory responses is, at least in part, mediated by changes in the peripheral code.

Poster session I Poster #115

Molecular insights into hermit crab olfaction

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For many species, behaviors such as foraging, mating or defense depend on detection of environmental chemical cues. The nature of these cues changes with the physical properties of each environment, e.g. water soluble compounds in aquatic, and volatiles in terrestrial habitats. In arthropods, and specifically insects, the dependence on chemical information has led to particular adaptations in sophisticated organs and a highly evolved olfactory and gustatory sense. Insects and crustaceans both belong to the Tetraconata phylum. Fossil records indicate that the last common ancestor of this phylum was aquatic when the hexapods split off and left the water environment in the late Silurian about 400 million years ago. Later crustaceans also succeeded in the transition from water to land in at least five independent lineages. Morphological and functional similarities like the connatural organization of olfactory organs and brain architecture provide a good example of parallel evolution in hexapods and crustaceans facing the same challenge, i.e. to smell in air. To retrace the evolution of the crustacean olfactory sense, we investigate and compare the antennal transcriptomes of terrestrial and aquatic hermit crab species, paying special attention to genes known to be involved in insect olfaction.

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Contributed talks I "Modulation of the olfactory system (Linnaeus Symposium)" Monday 25 June

Food-mediated regulation of chemoreceptor expression in *C. elegans*

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Plasticity in the expression of chemoreceptor (CR) genes may allow animals to modify their chemosensory responses in changing environmental conditions. The nematode *C. elegans* is an excellent system to dissect the mechanisms by which environmental signals are translated into appropriate changes in gene expression and behavior. Each *C. elegans* chemosensory neuron expresses multiple CR genes. A simple mechanism by which *C. elegans* can rapidly modulate their sensory behaviors in response to changing environmental conditions is via modulation of expression of subsets of CR genes in individual chemosensory neurons. Previously, we and others showed that the expression of CRs can be altered in response to neuronal activity and environmental cues. This provides a simple mechanism by which *C. elegans* can rapidly alter its sensory behaviors in response to changing environmental conditions. What are the neural and molecular mechanisms that underly plasticity in CR gene expression? We recently showed for the first time that CR gene expression in the olfactory neuron type, ADL, in *C. elegans* is modulated by the presence and absence of food. Using subcellular laser surgery and genetic tools, we showed that ADL functions as a food sensor to modulate CR expression. We also found that sensory inputs from food modulate CR gene expression through the neuropeptide Y receptor, NPR-1, signaling pathway. These results suggest that, in addition to the cell-autonomous function of ADL, neuropeptide release from ADL and peptidergic feedback possibly from interneurons tunes CR gene expression in ADL in response to food. We are currently exploring the neuropeptide ligands and neural circuits involved in food-mediated regulation of CR gene expression. We expect that our findings will not only yield insights into the mechanisms underlying CR expression in sensory neurons in other organisms, but also provide information regarding the basic mechanisms underlying behavioral plasticity.

Contributed talks II "Gustation" Monday 25 June

The (unfolded) mystery of starch detection in *Drosophila melanogaster*: tasteless but attractant.

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The understanding we have of insect taste perception is restricted to the detection of relatively simple chemicals. While starch, a polymer of glucose, is commonly found in the diet of *Drosophila melanogaster*, we do not know if and how flies are able to detect it. Using PER tests and binary food choice assays, we confirmed that starch is tasteless. In particular, in the choice assay, we found that starch (3%) is not consumed by flies. Interestingly, when starch is mixed with fructose (35mM), patches of fructose associated with starch are preferred over patches of fructose-only. One common hypothesis is that flies might be able to adjust their feeding according to the associated caloric reward. How such an association could be made between a post-ingestive information and a food with no particular taste was unclear to us. Results from Haj-Ahmad and Hickey (1982) indicate that feeding activities of flies induce a partial digestion of starch. Since the degradation of starch produces maltose, this suggests that starch-enriched food patches become sweeter than fructose-only patches. So as to test this hypothesis, we added an α-amylase competitive inhibitor, α-cyclodextrin (αC), in the food patches to prevent starch from being transformed. αC abolished the preference towards starch-enriched food patches. Similar results are obtained with *amy-null* flies. We also investigated if the taste system is involved in this process.

ΔGr64a[1] flies, that do not detect maltose, are unable to detect starch, whereas other taste-impaired mutants exhibited the same behaviour as control flies. The role of the "sweetness context" was also investigated. Taken together, these results indicate that flies pre-digest their food by releasing enzymes that dynamically change its taste. These results contribute to a better understanding of the subtle mechanisms by which insects perceive and interact with their environment.

Poster session I Poster #91

4D morpho-functional imaging of the honey bee brain

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We set up a two-photon microscope for in-vivo imaging of insect brains. First experiments focused on the primary olfactory centers, the antennal lobes, of the honey bee (*Apis Mellifera*). The system allows both 3D-tomographic measurements of the antennal lobes' morphology and highly time-resolved in-vivo calcium imaging of their neuronal activity.

Morphological data could be acquired down to 400μm penetration depth, allowing precise in-situ measurements of the glomerular volume. Functional imaging permitted recording of glomerular response maps to external odour stimuli with 20ms temporal resolution. The applied technique exceed by far the spatial and temporal resolution and the penetration depth of conventional imaging methods, minimizing in addition the photo-damage. This provides a new tool for insect neuroscience, allowing to investigate e.g. the role of subsurface glomeruli, dynamical odour coding, or optical studies of morphology and activity at the single neuron level.

Poster session I Poster #393

Central processing of nutritive and non-nutritive taste stimuli in young and middle-aged adults

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The neuroimaging literature suggests that the young adult brain can dissociate a sweet taste linked to a caloric value from a sweet taste devoid of energy content. However, little is known about central processing of natural and artificial sweeteners in the human brain during middle age. In the current study, we investigated fMRI activation of young and middle-aged adults during hedonic evaluation of a nutritive (sucrose) and nonnutritive (saccharin) sweet taste after a 12 hour fast. There were no differences in hunger ratings prior to the scan, or psychophysical ratings of intensity or pleasantness of either taste between the age groups. One-sample t-tests were run on activation to sucrose and saccharin separately for the young and middle-aged groups. Activation maps revealed significant clusters of activation in reward (dopaminergic midbrain, caudate nucleus) and prototypical taste regions (thalamus, insula) in the young and middle-aged adults in response to both the nutritive and nonnutritive sweet taste; however, activation was more robust for the young adults. Interestingly, while the nutritive sweetener elicited more widespread activation than the nonnutritive saccharin in the young adults, the middle-aged group demonstrated similar activation patterns to both tastes. These results suggest that there may be age-related changes in central processing of nutritive and nonnutritive sweet tastes during middle-age.

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Symposium 9 "Chemosensory initiated mating behaviour" Sunday 24 June

Deciphering chemical signals of birds: A brief history, current evidence, and promising future

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Birds employ colourful visual displays and elaborate song during breeding months. Compared to other vertebrates, however, the role of chemical communication has been largely overlooked, probably because overt behaviours linked with chemical signals are rare. Nearly 50 years after pioneering studies in avian anatomy and odour-mediated foraging, the chemosensory ability of birds is now, finally, generally accepted. Production of chemical substances is also widespread within Aves. The role of biogenic chemicalsignals has particularly emerged as an exciting new field in avian behavioural ecology. This reflects a rapid broadening of research focus within the past ten years, from olfactory navigation in select species, to chemical cues functioning in a wide-range of behavioural contexts and taxa as different as penguins, parrots and passerines. Key advances in social chemosignals include data from high latitude seabirds and the possible role of indirect and direct mechanisms of mate choice. Evidence in petrels (procellariiforms, Antarctic), for example, highlights inter-individual patterns of semiochemicals and forges potential links with the genetic compatibility of a mate. In auklets (alcidae, Arctic) odour concentration experimentally repels ticks, and new data correlates odour with sex and social rank. Evidence from these seabirds and other avian groups demonstrates definitively that avian chemical signals exist in the context of breeding. It also provides fertile ground for future studies to re-examine important topics in avian reproduction from the perspective of chemical signals, such as inter- and intra-sexual selection, or early chemosensory exposure during development.

Poster session I Poster #427

Candidate receptors for protein breakdown products in gastric endocrine cells

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Sensing of proteins and their breakdown products in the luminal content of the stomach is of particular importance for the regulation of digestive activities, including the release of gastrin and somatostatin, hormones which are of fundamental importance for controlling the gastric activities. The molecular basis for eliciting and tuning the release of these hormones according to the protein content in the gastric lumen is still elusive. Analysing the murine stomach for receptors capable to recognize protein breakdown products in the luminal content, it was found that the receptor types GPRC6A and CaSR which are proposed to be responsive to amino acids are expressed in the stomach; especially in the gastric antrum. Using immunohistochemical approaches a large population of GPRC6A- and CaSR-positive cells was visualized in the basal half of the antral gastric mucosa. Molecular phenotyping of the GPRC6A- and CaSR-immunoreactive cells revealed, that most of them contained the peptide hormone gastrin. A small population turned out to be immunoreactive for somatostatin. During the course of this study, it was found that the recently deorphanized receptor GPR92, which responds to protein hydrolysates and accordingly called peptone receptor, was also expressed in the gastric antrum. Phenotyping of the GPR92 cells revealed that GPR92-immunoreactivity was visible in many cells in the gastric antrum and it turned out that most of them contained gastrin. The finding that the amino acid responsive receptors GPRC6A and CaSR as well as the peptone receptor GPR92 are expressed in many if not all gastrin cells strongly suggests that all three receptor types are co-expressed in the same cells. These receptor types may contribute to responsiveness of the gastric endocrine cells to protein breakdown products.

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Poster session I Poster #237

Vapor-phase TRPM8 Agonists as Retronasal and Oral Cavity Stimuli

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Membranes of sensory terminals of trigeminal neurons present a range of ion channels that respond relatively selectively to environmental changes. One category is TRP channels, including TRPM channels, which differ themselves in which events or chemicals activate them, i.e., are agonists. Human sensory responses to vapor-phase trigeminal chemical agonists have been characterized as "irritation", " . . . ranging from freshness or tingling (e.g., in response to menthol) to burning or stinging (as elicited by ammonia or chlorine)." (Shusterman 2002). However, Parikh et al. (2009) reported that the TRPM8 agonist dl-menthol was identified as peppermint.

Hypothesis: 1) Some vapor-phase TRPM8 agonists elicit non-irritation descriptions. 2) Oral and nasal cavity trigeminal systems may provide different responses.

Methods: The TRPM8 agonists eucalyptol, geraniol, isopulegol, l-carvone, linalool, and dl-menthol, in vapor-phase, were presented oral-cavity-only (OCO) in discrimination trials, and both OCO and retronasally in identification (ID) trials.

Results: *Discrimination*: At room temperature maximum concentrations, OCO isopulegol and l-carvone were discriminated from blanks but OCO geraniol was not. However, when isointense the TRPM8 agonist could not be discriminated from blanks OCO by untrained participants. I*dentification*: With isointense stimuli, no OCO ID were found. Retronasal ID for l-carvone was spearmint, for geraniol, lemon, but retronasal isointense linalool, isopuegol, and dl-menthol received all available ID choices and did not differ from each other.

Conclusions: 1) The oral cavity trigeminal system may not permit ID of, or discrimination between, vapor-phase TRPM8 agonists. 2) With retronasal vapor-phase presentations the TRPM8 agonists geraniol and l-carvone receive non-irritation

ID that differ from other TRPM8 agonists. 3) Different retronasal TRPM8 agonists elicit different ID, indicating multiple receptor mechanisms.

Poster session II Poster #158

GABA(B) modulates the chemosensory functional map at the first synapse in the mouse accessory olfactory bulb

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The mammalian accessory olfactory system specializes in detecting non-volatile socially-relevant chemosignals. In the mouse, the initial detection is performed by vomeronasal sensory neurons (VSNs), which send their axons to the accessory olfactory bulb (AOB). The AOB is organized so that cells expressing the same receptor type co-innervate a region of neuropil called a glomerulus.

In the AOB, the glomeruli are all located at one face of the bulb, but are stacked on top of one another in a complex three-dimensional arrangement. We have mapped the functional organization of the glomeruli in response to stimulation by sulfated steroids, the largest-known class of ligands for VSNs. Using objective-coupled planar illumination (OCPI) microscopy with mice expressing GCaMP2 in VSNs, we obtained volumetric images of the VSN population activity in the AOB glomeruli.

To determine the presence of feedback on presynaptic representations, we measured the effect GABAergic neurotransmission on glomerular functional responses. Antagonism of presyanptic GABA(B) receptors relieved inhibitory feedback resulting in larger presynaptic responses. In addition to modulating activity within responsive glomeruli, GABA(B) antagonism altered the temporal dynamics of many responses . These results indicate that GABA(B) feedback onto these first synapses occurs during normal neurotransmission and shapes the chemosensory functional response map.

Contributed talks III "Mixed session" Monday 25 June

Crustaceans that colonized land: the troubles of evolving aerial olfaction when you had marine ancestors

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In addition to the ancestors of insects, representatives of five lineages of crustaceans have colonized land. Whereas insects have evolved olfactory sensory neurons that recognize specific airborne ligands, there is so far little evidence for aerial olfaction in terrestrial crustaceans. Therefore, we have asked the question whether terrestrial crustaceans have evolved the neuronal substrate for the problem of detecting far-field airborne chemicals. In the present study, we have compared the architecture of the central olfactory pathway in marine and terrestrial representatives of three independent crustacean lineages, the Anomura (hermit crabs), Brachyura (red crabs), and Isopoda (wood lice).

We show that conquest of land of Isopoda has been accompanied by a radical diminution of their first antennae and a concomitant loss of their deutocerebral olfactory processing areas. The same holds for the brachyuran representative that we examined, the endemic Christmas island red crab Gecarcoidea natalis. Its olfactory lobe and first antenna are much smaller than those of related marine species such as the green crab Carcinus maenas. Contrary, the land hermit crab Coenobita clypeatus and the giant robber (coconut) crab Birgus latro as terrestrial representatives of the Anomura have greatly enlarged primary and secondary olfactory centers as compared to their marine relatives.

If we assume that maintaining nervous tissue is very costly, unused brain structures will erode away quickly in evolution. Hence, the size of central olfactory processing areas to a certain extent reflects the animal's ability to detect chemical cues. We conclude that for an arthropod olfactory system an evolutionary transition from a marine to a terrestrial ecology is not an easy task to accomplish. For terrestrial Crustacea, so far only Anomura seem to have been successful in this transition. Supported by the Max Planck Society and HA 2540/8.

Poster session II Poster #416

Tracking spoiled meat using an electronic nose based on support vector machine classification

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Over the last few decade, quite worth mentioning efforts have been made for developing sensor system also known as an electronic nose to facilitate the odor analyses. With the invent more sophisticated electronic chemical gas sensor, the electronic nose has been introduced in the food industry for different applications such as quality control, process monitoring, freshness evaluation, shelf-life investigation and authenticity assessment. In this study, the aim is to develop an electronic nose for monitoring the meat stocked inside a house at room temperature. Electronic nose analyses the samples of beef and fish and applies a classifier named support vector machine (SVM) to identify the meat creating malodor. To evaluate the performance we captured beef and fish meat for 4 days and monitored it. The results indicate that SVM classifier exhibits good generalization performance and enable accuracy rate of almost 94.5 % for both beef and fish. This means that SVM is an effective pattern classification technique for meat identification for the electronic nose.

Poster session II Poster #380

Assessment of expression of a presurgically conditioned taste aversion to NaCl and concentration dependent licking of sucrose and quinine after bilateral damage to the gustatory cortex in rats

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It has been reported that damage to gustatory cortex eliminates the retention of a presurgically conditioned taste aversion. Interestingly, unconditioned taste preferences and aversions, as assessed in intake tests, have been reported to be normal after gustatory cortex lesions in rats. Here we presurgically paired 15-min ingestion of 0.1 M NaCl with either LiCl (2.0 mEq/kg, IP, n=22) or saline (2.0 mEq/kg, IP, n=22) on two occasions. Rats then received either bilateral injections of ibotenic acid (20 mg/ml, 0.18 µl, n=28) or PBS (0.18 µl, n=16) targeting the center of the gustatory cortex (in mm: +1.3 AP, ±5.2 ML, -6.8 DV). Surprisingly, LiCl-injected rats histologically evaluated to have sustained extensive bilateral damage to the gustatory cortex (n=5-6) displayed no impairment in retaining a specific aversion to NaCl as assessed by a 30-min brief access test (repeated 10-s trials) with H_2O, 0.03, 0.1, and 0.3 M NaCl and KCl or in a subsequent 48-h 2 bottle preference test. These rats were also tested in a fasted state for their responsiveness to various concentrations of sucrose (S) and, in a water-deprived state, of quinine hydrochloride (Q) in a series of brief access tests (10-s trials, 30-min sessions). The concentration-response relationship for S and Q did not significantly differ between rats (w/ at least 2 trials/conc/session) with histologically confirmed bilateral lesions (S, n=10; Q, n=11) and those with control injections (S, n=11; Q, n=15). Apparently, rats with extensive damage to the gustatory cortex display normal concentration-dependent affective responses to S and Q even when postingestive factors are minimized. However, in contrast to prior reports in the literature, these same animals were able to retain and express a presurgically conditioned taste aversion. The disparity between our findings and others remains to be understood but could relate to the exact topography and locus of the neural damage across studies. NIH R01-DC009821

Poster session II Poster #202

Axonal growth patterns of sensory neurons in a developing olfactory system

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The vertebrate olfactory system undergoes continual neurogenesis. Throughout the lifetime of an organism, new sensory neurons are generated by olfactory stem cells. These neurons extend axons over long distances towards the anterior telencephalon establishing synaptic contacts with second-order neurons in specific target regions, so-called glomeruli. In rodents, sensory neurons normally project only into one specific glomerulus of the olfactory bulb. Here, we investigated axonal growth patterns in the developing olfactory system of larval *Xenopus laevis*. We used electroporation to introduce fluophore-coupled dextrans or plasmid DNA, encoding fluorescent proteins, into cells of the olfactory system. Electroporation of the whole olfactory organ revealed the main sensory projection fields within the olfactory bulb, namely the accessory olfactory bulb and a lateral, intermediate and medial glomerular cluster in the main olfactory bulb. In contrast to the accessory olfactory system, the main olfactory system undergoes complete restructuring during metamorphosis. The axonal branching patterns of sensory neurons originating from both, the vomeronasal and main olfactory epithelium, were investigated by staining of fewer or single cells. Synaptic connections were clearly visible as tufted axonal endings. Axons of sensory neurons of the main olfactory epithelium showed a branched morphology and regularly terminated in multiple glomeruli. Also sensory neurons of the vomeronasal organ showed axonal branching in the olfactory bulb. Different developmental stages were investigated to address the question if bifurcated axon growth is a general feature of the developing olfactory system and how connectivity is reshaped during maturation of the system.
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Poster session I Poster #27

Does 20-hydroxyecdysone induce modulation in the olfactory circuitry of *Spodoptera littoralis*?

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During mating, different signal cascades are triggered in animals that induce behavioural changes. One of the main hormones involved in egg maturation and induction of oviposition in insects is 20-hydroxyecdysone (20-E). Our group recently found that mating triggers a behavioural switch in the moth *Spodoptera littoralis* by inducing differential changes in sensitivity of the antenna and antennal lobe (AL). Unmated females prefer odours from lilac flowers over odours from cotton plants, whereas mated females prefer the opposite. The neurophysiological mechanism underpinning this switch in behaviour and olfactory sensitivity is the topic of this study. Here we investigated the neurophysiological processes of the observed switch of *S. littoralis* females, primarily focusing on the role of 20-E. We hypothesized that 20- E acts directly or indirectly on the antenna and AL by changing its sensitivity to volatiles from lilac flowers and cotton leaves. We performed pharmacological manipulations in moths to reproduce the observed fluctuations of 20-E in virgin and mated moths and tested the antennal sensitivity and antennal lobe activity using EAG and calcium imaging techniques, respectively. The results and implications for *S. littoralis* females will be discussed.

Poster session I Poster #203

Presentation of contextual odor cues during sleep selectively destabilizes fear memories

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Recent research has demonstrated that new episodic memories can be selectively manipulated during sleep, via night-time delivery of olfactory contextual cues previously introduced during daytime learning (Rasch et al., 2007). However, whether non-episodic, emotional memories can also be manipulated during sleep is unknown. Given the intimate anatomical and functional connections between olfactory and limbic systems, we sought to apply odor as a contextual cue to manipulate classically conditioned fear memories. Human subjects (n=15) participated in a contextual conditioning paradigm, in which they acquired a fear response to stimuli associated with one of two odorant contexts. One odorant was then re-presented during sleep (thus presumably reactivating the fear memory for stimuli associated with this odorant). Fear responses to stimuli were re-assessed after awakening. Responses to stimuli that had been reactivated via odorant presentation during sleep were compared to non-reactivated stimuli, as measured by physiological recordings (i.e., skin conductance) and fMRI. Results indicated that physiological fear responses to re-activated stimuli were significantly diminished in comparison with non-reactivated stimuli, and decreased fear responses were accompanied by reduced neural activity in hippocampus and insula, on a subject-by-subject basis. Furthermore, duration of odorant presentation during sleep was significantly correlated with decreased physiological responses as well as decreased activity in perirhinal cortex (a key region in fear conditioning). Our results demonstrate a destabilization of fear memory for stimuli re-activated during sleep via contextual odor cues. These findings have potential clinical applications as well as theoretical significance for understanding olfactory processing during contextual fear conditioning and sleep. Research in this presentation is supported by the NIH (NIDCD 1R01DC010014, NINDS 5T32NS047987, NIMH 1F32MH091967).

Poster session II Poster #28

Tracing the neuronal correlate of olfactory flower perception in *Manduca sexta*

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Categorizing environmental information is a principle task of an animal's nervous system. Pollinators such as the hawkmoth *Manduca sexta* appear to innately categorize flowers into suitable and unsuitable nectar sources in order to increase their foraging efficiency. More than in other pollinators, such as bees or hummingbirds, the foraging behavior of *Manduca* seems to be predominantly guided by odors. In this context, it is of particular interest that the chemical composition of the floral volatiles emitted by different hawkmoth-pollinated plants, does not reveal any patterns that would universally distinguish nectar sources of different suitability to the hawkmoth, indicating a more perceptual categorization of the floral odor.

The aim of our project is to determine whether the primary olfactory centre of the *Manduca* brain, the antennal lobe, can categorize flower bouquets according to their suitability for nectar feeding, in spite of the chemical similarities in odor composition. Through a direct use of the full head-space of several hawkmoth- and bee-pollinated flowers from the *Nicotiana* family, we will be able to compare the calcium dynamics of the *Manduca* antennal lobe after presenting these two groups of ecologically relevant stimuli. Additionally, we will establish complementary methods of extracellular recording, which will allow a combined use of calcium-imaging and electrophysiological techniques. Utilizing these methods we will investigate both spatial and temporal components of odor coding simultaneously with in the same animal animal.

Our results will contribute to the general understanding of pollination ecology, and will also allow us to begin to answer the question of "whether important categorization tasks might already be performed within the antennal lobe of *Manduca sexta* through spatial and temporal coding".

Poster session II Poster #430

Emulsifying properties of oolong tea

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Oolong tea is a traditional Chinese tea and now is a popular beverage widely consumed in the Far East. Green, oolong, and black teas all come from the leaves of the *Camellia sinensis* plant. Oolong tea is set aside and maintained in stringently controlled highly sensitive environments to oxidize at its own pace, i.e. semi-fermented product. The tea is also known as weight loss tea which has been scientifically proven to increase metabolism and fat oxidation. Besides the physiological action, refreshment of oiliness in oral cavity is an attractive property of oolong tea in high-fat meal. We showed, in the last ECRO, that the oolong tea reduced the oiliness in oral cavity more than the water with the sensory evaluation and decreased interfacial tensions of water/oil more than the water and green tea In this study, another physical properties related to remove oiliness from oral cavity were analyzed using the teas and water.

Particle size analysis: The particle size of emulsion effects on the texture and creates a unique feeling on the tongue. For the preparation of emulsion, soya-bean oil and water were mixed with high blend mixer at 19,000 rpm for 3 min. followed by homogenation with the ultra-sonication. The particle size tended to decrease in oolong tea/oil emulsion but there was no significant difference from emulsions of green tea/oil and water/oil.

Emulsion stability: An appearance of emulsion is the index of the emulsion stability. The phase separations of water/oil and green tea/oil were observed quickly just after sample preparation. And the amounts of separated oil were increased in a time- dependent manner. Emulsion of oolong tea/oil showed the better stability than those of water/oil and that of green tea/oil.

These results suggest that higher affinity of oolong tea to oil than other beverages supports to taking oil from oral cavity and keeping it until gastrointestinal tract without re-adhesion.

Symposium 2 "Coding of taste across mammals: from the tongue to the cortex" Saturday 23 June

Coding of taste from the tongue across mammals as revealed by the phylogeny

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Molecular data show that taste sensations already on the level of the taste buds are divided into categories, because the taste receptor cells are not uniformly responding to every taste stimulus. Instead, their responsiveness generally falls into the categories of sweet, sour, umami, salty and bitter, that is what humans call taste qualities. This separation is upheld in the taste buds and continues in the nerve fibers that transmit the sensations from the taste buds to medulla oblongata. The closest parallel with human taste qualities is reported in chimpanzee, whose taste fibers conform almost to hundred percent with the human division in sweet, umami, sour, salty and bitter. This relationship is somewhat less obvious in Old-world monkeys represented by macaques, but is still very evident in New-World monkeys represented by, for example, marmosets also known as tamarins. As one proceeds along the phylogenetic scale from primates to rodents and ruminants, the connection with the human taste qualities and the taste of the compounds, which we chose as representative of these diminishes and is lost in ruminants, such the cattle. Nevertheless, in each species there are always two groups of taste fibers, one which stimulates to intake and one which causes rejection.

Poster session II Poster #310

The olfactory sniff-response as a diagnostic marker for autism

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Whereas parental reports of behavior in children with autism routinely include descriptions of olfactory symptoms, typically hypersensitivity, only few controlled studies have measured olfactory performance and preferences in individuals with autism. Olfaction, however, may be a particularly well-suited modality for testing individuals with autism because it includes a non-verbal measure of perception, namely the sniff-response. Typically, pleasant odors are sampled using vigorous sniffs, and unpleasant odors are sampled using mild sniffs. Thus, sniff-measurements provide a non-verbal measure of olfactory perception. Here we set out to test the hypothesis that the sniff-response may provide a non-verbal diagnostic marker for autism. We developed a device that delivers odors and measures the sniff-response, all through a child-sized nasal canula. The child's attention is maintained through an on-screen cartoon, while pleasant and unpleasant odorants are administered, and the sniff-response recorded. The ~8 minute interaction does not entail any verbal response, or active reply, and can therefore be applied in pre-verbal or poorly interacting children. We predict that the odorant-specific sniff-response will differ in children with autism compared to typically developing children. To date, we have piloted 7 typically developing children, 6 boys and one girl, ranging in age from 18 to 67 months. In 6 of the 7, odor administration drove a sniff modulation ($t(6) = 2.49$, $p < 0.05$). The device is now being placed in the major diagnostic facility for autism in Israel, and this presentation is expected to include data from a large sample of children with autism.

Poster session I Poster #29

Unravelling mosquito taste: a beginning.

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We investigated feeding-related decisions in *Aedes aegypti* (L.) by presenting adults with simple diets of paired gustatory stimuli conveying information concerning energy content, nutrient richness, osmotic balance and food toxicity in a two-diet matrix assay. Using this assay, mosquito diet-choice was tested between two equimolar sucrose 'driver' solutions, with one presenting various concentrations of another potential feeding cue 'test' compound (e.g. amino acids, salts or alkaloids). A systematic characterization of diet selection behaviour of male and female *A. aegypti* is presented for 27 putative feeding cues potentially involved in both blood and nectar/honeydew feeding.

The molecules that confer such selectivity and sensitivity to contact chemoreceptive neurons constitute a superfamily of highly divergent seven-transmembrane domain proteins (i.e. <20% amino acid similarity), and are known as gustatory receptors (Grs). This high degree of divergence amongst chemoreceptors may be, in no small part, responsible for the range and plasticity of mosquito feeding behaviours. While the ligands have been predicted for a few mosquito Grs, for the majority of Grs the ligands are still unknown. On the way towards de-orphaning these Grs, we have now completed the tissue and sex expression profile of *Ae. aegypti grs*, are investigating the ligand profiles of differentially expressed Grs using *in vivo* and *in vitro* heterologous expression techniques.

Here we provide a solid foundation for future research into taste, not only in mosquitoes, but also in insects in general. Investigations into areas such as the molecular basis of gustatory behavioural plasticity in response to physiological state will be made possible. In mosquitoes, this research will also lead to expanded options for biological control of these highly dangerous disease vectors and aid researchers in accurately determining vectoral capacity.

Poster session II Poster #30

Wnt5 and Drl regulate dendritic targeting in *Drosophila* olfactory map formation

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The orderly termination of olfactory axons in the olfactory bulb is critical for animals to perceive and discriminate smells. Yet, after many years of research, the developmental origin of the olfactory map remains a mystery. The lab of Liqun Luo showed that in *Drosophila*, the olfactory map forms through targeting of the projection neuron (PN) dendrites in the nascent antennal lobe (AL). It was hypothesized that the dendrites were responding to pre-existing positional cues secreted by guidepost cells. However, what the cues are, or whether guidepost cells exist remain to be clarified. We have shown previously that disruption in the expression of Wnt5, a secreted protein, led to the severe derangement of the olfactory map. We now present evidence that Wnt5 is expressed in a gradient in the nascent AL and disruption of the Wnt5 expression led to the aberrant targeting of the PN dendrites and a corresponding disruption of the glomerular map. Our finding makes Wnt5 the first known guidance cue for PN dendrites, and the earliest pattern element in the AL. We also show that Drl, the Wnt5 receptor, is expressed by PN dendrites projecting to regions of high Wnt5 concentration, and loss of Drl also disrupted dendritic targeting. Unexpectedly, mutations in *wnt5* alleviate the dendritic defects caused by loss of *drl* function. This result, together with the overlapping expression of the Wnt5 and Drl proteins, suggests that Wnt5 acts as a repulsive signal for PN dendrites and that Drl downregulates Wnt5 signaling. Finally, we show that the domains of Wnt5 are linked to a cluster of cells located some 50 μm lateral to the AL. The cells strongly express Wnt5 and extend long $Wnt5^{+}$ processes to the ipsilateral AL, where the fibers appear to branch in the regions of high Wnt5 staining. We propose that these mysterious cells set up the domains of Wnt5 expression in the nascent ALs and that they are the sought-after olfactory guidepost cells, a developmental template of the olfactory map.

Poster session I Poster #31

Opposite effects of external appetitive olfactory stimuli and intrinsic peptide– controlled suppressing motivation on feeding behavior in blowfly, *Phormia regina.*

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Most of animal behaviors are affected not only by external stimuli via sensory systems but also by intrinsic motivation under neuronal and/or hormonal regulation. It is known that both gustatory and olfactory systems are involved in appropriate motor pattern formation for feeding behavior, which is also internal condition dependent. Neuronal or hormonal peptides concerning feeding behavior have been discovered and investigated their roles related with the internal condition. Here, focusing on the novel peptides (dRYamides-1 and 2) found in *Drosophila melanogaster,*, we compared their suppressive effects on the feeding behavior of the blowfly, *Phormia regina*.

Proboscis extension response (PER) is the indicator of feeding behavior that is generally controlled by the phagostimulative tastant. The behavioral tests showed that palatable odor increase not only the rate of PER and also food intake amount in blowfly, *Phormia regina*. They usually have detected the appetite odor like mushroom alcohol (1-octen-3-ol) by accessory olfactory organ, maxillary palps.

Next, we determined the effective dose of these peptides on the feeding behavior of the blowfly. We injected various amounts of these peptides, respectively, and found that 10pmol peptide injection clearly decreased the PER occurrence. Third, we investigated the time course of appearance of their effects on the PER occurrence; The suppressive effects of dRYamide-1 on the PER occurrence appeared more rapidly than dRYamide-2.We further treated the flies with peptide injection and olfactory stimulation with 1-octen-3-ol, and investigated behavioral changes in feeding. Under high dose administration of peptide, olfactory stimuli didn't work efficiently.

These results suggest that these peptides work as the appetite-suppressant in their nervous system through different pathway to realize appropriate motor patterns on blowfly feeding behavior, and it did worked as counteractant to extrinsic sensory cues.

Poster session I Poster #169

Morphology of the lateral nasal gland duct in domestic chicken *Gallus gallus domesticus*

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The domestic chicken (*Gallus gallus domesticus*) possesses a paired tubular organ called the lateral nasal gland duct (LNGD) at the base of the nasal septum. This organ communicates rostrally with the nasal cavity, and caudally tube ends blindly. The gross anatomy of this organ appears to be similar to the vomeronasal organ of other vertebrates. However, there is little information regarding the function of the LNGD. Thus, the objective of this study was to elucidate the morphological features of the LNGD to reveal its role. HE staining showed that the rostral region of the LNGD lumen was elliptically shaped in coronal section, and the caudal part of the lumen was oblong crescent shaped. Several blood vessels and paired nerves could be distinguished in the LNGD surroundings. The enclosed cartilage and grandular tissue were barely observable. PAS-Alcian blue staining revealed several lumps in the apical portion of the epithelium, which was PAS positive. Immunohistochemical staining revealed that several types of cell expressed the G protein α subunit Gαolf/s in the epithelium. Furthermore, some cells also expressed trace amine associated receptor 2 and neural cell adhesion molecule, respectively. Electron microscopy showed that the epithelium consists of olfactory sensory-like, supporting-like, and basal-like cells. In addition, apical portions of the olfactory sensory-like cells were united by the tight junction. Taken together, the LNGD of the domestic chicken has been unclear. However, our observations suggest that the epithelium of the LNGD is representative of the olfactory organ, rather than the duct of the secretary gland.

Poster session II Poster #400

Endogenous gustatory response and properties of immortalized human taste cell lines from lingual epithelium

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Stably proliferating human taste cell lines will be a powerful tool to gain new insights into human taste reception and signal transduction mechanisms as well as to develop new technologies for industry applications. We used biopsy samples from human lingual epithelium containing taste buds from fungiform, foliate and circumvallate papillae to obtain primary human taste cells. Isolated cells were transduced with Adenoviruses carrying immortalization gene cassettes comprising previously established immortalization genes. We obtained several proliferating cell populations suitable for characterization using molecular biology tools as well as for evaluation with HTS-compatible cell-based assays to quantitatively assess endogenous gustatory response of human taste cells.

Two cell lines were chosen for gene expression analysis of select taste reception and signaling genes. Despite immortalization treatment, both cell lines revealed gene expression profiles resembling those of differentiated taste cells as well as those of progenitor cells residing in the taste bud. RT-PCR analysis of genes encoding taste receptors, ion channels and other potentially relevant factors revealed for instance that 15 of 25 human TAS2R bitter taste receptor genes as well as the Oxytocin receptor gene are expressed. Intriguingly, Fluo-4 Calcium assays revealed that our

immortalized cell lines responded strongly with Calcium signaling when challenged with appropriate bitter compounds or Oxytocin. However, the cells were insensitive to compounds representing other basic taste modalities such as sweet, umami, salty and sour. These experiments provide evidence that we successfully established proliferating human taste cell lines, which maintain their endogenous programming and dedication to bitter taste perception.

Poster session I Poster #171

The tissue distribution of a putative pheromone precursor, felinine and its precursor, 3-methylbutanol-glutathione, in the domestic cat

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Felinine (2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoicacid) is a sulphur containing amino acid that is excreted in the urine of domestic cat and its near members in felidae species. Felinine is a precursor of a cat specific odorant, 3-mercapto-3-methyl-1-butanol (MMB), that is a putative pheromone in cats. Previous studies identified 3-methylbutanol-glutathione (MBG) as a felinine precursor in the serum. This finding suggests that MBG is formed via a glutathione S-conjugation reaction between glutathione and isopentenylpyrophosphate (IPP) in the liver and a cat-specific glutathion-S-transferase for IPP is involved in the reaction, because IPP is an intermediate of cholesterol biosynthesis from mevalonic acid and present in not only cats, but also other mammals. However, little is known about the biosynthetic pathway of MBG in vivo. In this study, we examined the tissue distribution of MBG and felinine to elucidate biosynthetic pathway of MBG. We developed an optimized LC–ESI-MS protocol which allowed precise quantification of felinine and MBG in tissue extracts of cats. LC-MS detects m/z 394.164, a molecular ion of MBG in not only serum and liver but also various tissues such as muscle, spleen, and stomach. In addition to MBG, a molecular ion of felinine (m/z 208.100) was also detected in the serum and the various tissues. MS analysis also demonstrated that the cat bile also contained MBG and felinine. Therefore, we analyzed gastrointestinal contents and found that felinine is contained in the contents and feces. GC-MS of the headspace gas above cat feces detected a felinine derivative, MMB. These results suggest that MBG is produced in not only liver but also other organs, the degradation of MBG occurs in not only renal tubules but also intracellular compartments of each organ, and feces odor with MMB may play an important role in the chemical communication of cats.

Poster session II Poster #172

Odor exposure during olfactory epithelial regeneration leads to enhanced representation of receptor neurons.

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The potential benefit of odor exposure therapy for patients with smell loss has been reported, but the basis for this has not been shown. The repopulation of olfactory neurons after epithelial damage is dynamic and partly influenced by the environment. Previous studies have shown that olfactory neuron stimulation provides a competitive advantage over non-stimulated neurons for survival and quantitative representation throughout the epithelium during development. We have previously found that the relative numbers of specific receptor neuron types vary from normal after recovery from epithelial damage using methyl bromide (MeBr). We hypothesized that the limited odorant environment of laboratory housing imparts an unnatural competitive advantage for certain types of olfactory neurons and that neuron survival could be artificially enhanced with repetitive exposure to an activating odor. We used several different strains of OR-targeted transgenic mice and exposed them to odorants known to activate c-Fos expression in the marker-tagged neurons. In an enclosed, constant-flow chamber, mice were exposed to cycles of odor for 2 minutes followed by air washout for 3 minutes for a total of 4 hours/day, 5 days/week, for 8 weeks following MeBr-induced epithelial damage. Counts of

specific olfactory neurons taken from sections obtained through the entire nasal cavity were compared with those from mice exposed to air or to a non-activating odorant. We found that specific odorant exposure during recovery from MeBr lesioning of the epithelium increased the number of targeted olfactory receptor neurons as compared to control. Our results support the hypothesis that odorant evoked neuron activity competitively enhances cell survival resulting in increased representation in the olfactory epithelium which, in turn, lends support for odor therapy as a treatment modality in patients with loss of smell.

Poster session I Poster #401

Taste responses in PRIP-1 and PRIP-2 double knock-out mice

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PRIP (Phospholipase C-related, but catalytically inactive protein, comprising type 1 and 2) was a novel inositol 1, 4, 5-trisphospate (IP_3) binding protein. It has number of binding partners, including GABARAP ($GABA_A$ Receptor Associated Protein), and plays important roles in trafficking to plasma membrane of $GABA_A$ receptor. In taste cells, IP3 binds to IP_3 receptor (IP_3R3) and IP_3R3 is a principal mediator of sweet, bitter, umami taste perception. GABA is a candidate neurotransmitter in taste buds, and precise role of GABA in taste buds remains unclear. Recently it has been reported that PRIP-1 and PRIP-2 double knock-out (DKO) mice show enhanced food intake as compared with wild-type (WT) mice. In DKO mice, subunit expression of $GABA_A$ receptors were altered in the brain. In this study, to investigate potential involvement of PRIP in the gustatory system, we examined the mRNA expression of PRIP-1 and PRIP-2 in fungiform taste buds (FP), circumvallate taste buds (CV) and epithelial tissues (ET) by RT-PCR and recorded chorda tympani (CT) and glossopharyngeal (GL) nerve responses to various taste stimuli from DKO mice and WT mice. RT-PCR on isolated taste buds showed that PRIP-1 and PRIP-2 were expressed in the FP and CV but not in the ET. As compare with WT mice, DKO mice tend to show increased responses to sweet, sour and salty compounds and decreased responses to bitter compounds in the CT nerve. Responses to umami compounds in both CT and GL nerves were not clearly different between DKO and WT mice. These results suggest that PRIP may be involved in mouse taste nerve responses. Further studies are needed to elucidate the function of PRIP in taste bud cells.

Poster session I Poster #431

Effect of odor on anaerobic performance in college athletes

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Previous studies suggest smelling odorants such as peppermint and sandalwood enhance athletic performance in activities ranging from pedaling on a stationary bicycle to weight lifting. In these studies, the activity performed was either mostly aerobic (such as riding a bike) or strength based (such as weight lifting). In the present study it was hypothesized that smelling these same odorants would improve performance during an anaerobic activity requiring agility, coordination, strength and speed. To test this hypothesis, members of the St. Lawrence Alpine Ski Team performed 5 blocks of clockwise and counterclockwise hex jumps, a common training activity, while smelling sandalwood, peppermint or distilled water. In each block, these three olfactory treatments were presented, one at a time, to the subjects by gauze pads taped to the upper lip. In a 15.5 second exercise, subjects were, on the average, half a second faster when smelling either peppermint or sandalwood compared to when smelling the odorless control ($p<0.05$). Although, as expected, the exercise resulted in an increase in heart rate, the increase was not different among the three olfactory treatments ($p>0.05$). In addition, the number of errors made was not affected by olfactory treatment ($p>0.05$). These results are consistent with the hypothesis that performance in an anaerobic activity requiring agility, coordination, strength and speed can be improved by smelling either peppermint or sandalwood. Perhaps this improved performance occurs because subjects are better able to focus on the task at hand.

Poster session II Poster #264

Behavioral significance of natural odor mixture components in the human brain.

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Natural odors are inherently complex in that they are often mixtures of dozens of unique molecular components. Given that natural odors are powerful cues for guiding behavior, the olfactory system provides an elegant experimental framework for understanding whether the brain encodes behaviorally salient information using elemental representations of discrete odor components or configural representations of the whole odor. Here we used a gas chromatography/mass spectrometry system to identify 14 molecular components of peanut butter odor. These components were presented, along with peanut butter odor and a control food odor, to human subjects during fMRI scanning both before and after they ate peanut butter to satiety. Pleasantness ratings for the peanut butter odor were significantly reduced from pre- to post-satiety compared to the non-sated food odor, consistent with sensory-specific satiety. This behavioral effect was mirrored by a selective response decrease to the sated food odor in orbitofrontal cortex (OFC), a region previously implicated as a key substrate for mediating sensory-specific satiety. Two pyrazines, which comprised 6/14 peanut butter components, elicited significant neural effects similar to those evoked by the mixture. In general, the pyrazines as a group evoked stronger satiety-related effects than other components. Notably, the amount of satiety-related signal change in OFC evoked by a component was directly related to how similar in quality that component was rated to peanut butter odor ($p < 0.005$). These results indicate that both physical and perceptual attributes of odor mixture components may contribute preferentially to the behavioral significance of the mixture itself, and raise important questions regarding elemental versus configural models of olfactory information processing.

Poster session I Poster #265

Time-frequency analysis of chemosensory event-related potentials to characterize the cortical representation of odors in humans.

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Introduction and aim: The recording of olfactory and trigeminal chemosensory event-related potentials (ERPs) has been proposed as an objective and non-invasive technique to study the cortical processing of odors in humans. Until now, the responses have been characterized mainly using across-trial averaging in the time domain. Unfortunately, chemosensory ERPs, in particular, olfactory ERPs, exhibit a relatively low signal-to-noise ratio. Hence, the current usefulness of the technique remains limited. We hypothesized that the relatively low signal-to-noise ratio of chemosensory ERPs could at least in part be due to temporal jitter. For this reason, we used a time-frequency analysis based on the wavelet transform to reveal EEG responses that are not strictly phase-locked to the chemosensory stimulus. We hypothesized that this approach would significantly enhance the signal-to-noise ratio of the elicited responses because, as compared to conventional time-domain averaging, (1) it is less sensitive to temporal jitter and (2) it can reveal non phase-locked EEG responses such as event-related synchronization and desynchronization.

Material and methods: EEG responses to selective trigeminal and olfactory stimulation were recorded in 11 normosmic subjects. A Morlet wavelet was used to characterize the elicited responses in the time-frequency domain.

Results: The time-frequency approach markedly improved the signal-to-noise ratio of the obtained responses, in particular, following olfactory stimulation. Furthermore, the approach allowed characterizing non phase-locked components that could not be identified using conventional time-domain averaging.

Conclusion: By providing a more robust and complete view of how odors are represented in the human brain, our approach could constitute the basis for a robust tool to study olfaction, both for basic research and clinicians.

Poster session II Poster #266

Enhancement of trigeminal chemosensory function in congenital anosmia and its contribution to the perception of odors.

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Background and purpose: The majority of odorants activate both olfactory and trigeminal systems, and both systems are thought to contribute to the perception of odors. It is well known that, as compared to controls, patients suffering from congenital anosmia exhibit enhanced trigeminal responses, suggesting that trigeminal chemosensory input constitutes a unique way for people suffering from congenital anosmia to perceive odorants. The aim of this study was to investigate the contribution of the trigeminal system to the perception of odorants in congenital anosmia.

Materials and methods: We retrospectively reviewed 25 cases of patients suffering from congenital anosmia. Chemosensory function was investigated using psychophysical testing as well as the recording of trigeminal chemosensory event-related brain potentials (CSERPs). A structural MRI was also performed to evaluate the morphology of olfactory pathways. Results were compared to those obtained from 19 patients suffering from acquired anosmia (15 post-traumatic and 4 post- infectious) and 18 healthy controls.

Results: The magnitude of the P2 wave of trigeminal chemosensory ERPs was significantly enhanced in congenital anosmia vs. healthy controls, and its latency was delayed. We also found a significant correlation between the psychophysical assessment of olfactory function and the magnitude of trigeminal CSERPs. This correlation was absent in acquired anosmia and healthy controls. Conclusion: Our results indicate that the chemosensory function of the trigeminal system is enhanced in congenital anosmia, as compared to acquired anosmia and healthy controls.

Symposium 9 "Chemosensory initiated mating behaviour" Sunday 24 June

The interaction between sex pheromones and genetic identity signals in rodents

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Scent signals play an integral role in mediating reproductive interactions in rodents, priming reproductive physiology as well as allowing animals to locate individuals of the opposite sex and to assess their attractiveness as mates. Many androgen-dependent pheromones have been identified in mice that prime female reproductive physiology when detected through specific vomeronasal receptors. While such pheromones appear to have generalized effects on stimulating readiness to mate, females are also highly selective in their choice between individual males. In house mice, males in particular invest very heavily in a set of highly polymorphic major urinary proteins (MUPs) that bind urinary volatiles and provide long-lasting signals in scent marks. Investment in these specialized communication proteins increases strongly under competitive pressure along with the rate of scent marking, increasing the strength of both volatile and involatile signal components that advertise individual competitive ability and location. Individual-specific MUP patterns and their bound volatiles provide a genetically encoded signal of individual identity, heterozygosity and kinship that allows females to identify males of high competitive ability and to avoid inbreeding. Attraction to specific males is coordinated by the male sex pheromone darcin, an atypical MUP that induces instinctive female attraction to spend time near male scent. Importantly, darcin also induces a very rapidly learned attraction to the associated airborne odour signature of that particular individual male and to his location. This pheromone-induced learning provides strong reinforcement of attraction to specific males and is likely to underpin the ability of females to remember and select preferred mates from those available, according to both quality and genetic compatibility.

Poster session II Poster #26

Neuronal encoding of gustatory and olfactory information in the tobacco budworm moth *Heliothis virescens*

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Insects are favourable model organisms for elucidating the neural mechanisms underpinning encoding and perception of gustatory and olfactory information. We have investigated physiological and morphological characteristics of higher order olfactory and gustatory neurons in the moth Heliothis virescens by the use of intracellular recordings. Recorded neurons were fluorescently stained, digitally reconstructed, and then integrated into a standard 3-dimensional brain atlas of Heliothis virescens. Aiming to understand the contributions of single neurons to the representation and differentiation of complex odor mixtures, we have identified odor tunings of single cells and compared response strengths and temporal characteristics of neuronal responses to complex odor mixtures and their single constituents . Our findings suggest a combinatorial encoding strategy where complex mixtures are uniquely represented by some cells, and where temporal response characteristics partakes in differential encoding of odor identities. Analyses of temporal features also allowed us to reveal differences between the response properties of phagostimulants and phagodeterrents in higher order gustatory neurons. Response profiles further showed grouping of neurons with regards to integration of taste qualities mediated by different appendages.

Poster session II Poster #394

Source analysis of electromagnetic-encephalography acquisitions in response to gustatory stimulation

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The identification of the primary gustatory cortex in humans is still under debate. Neuroimaging studies indicate Insula and overlying operculum as the best candidates (Iannilli et al. 2012, Rolls and Scott 2003) but the exact position inside this brain region is not entirely clear (Small 2010). Moreover, inconsistencies appear when comparing results from studies using functional magnetic resonance imaging (FMRI), and gustatory even-related-potentials (gERP), or gustatory event-related magnetic fields (gERMF). FMRI indicate activations in the anterior part of the Insula and frontal operculum (Schoenfeld et al. 2004), while gERPs and/or gERMF indicates activations at the transition between the parietal operculum and insula in its posterior part (Murayama et al. 1996, Ohla et al. 2009). Here it is important to note that for gERPs and gERMF the stimulus onset must be well controlled in time and space to evoke a recordable brain ERMF. Recently a new generation of computer-controller gustometers came forward (Gu002/GM05, Burghart, Wedel, Germany) that has been demonstrated to elicit classical gERPs (Singh et al. 2011). In order to localize the site of activation a multi-channel set up is required, in fact in this condition an inverse solution equation can be implemented to compute the forward problem and find the electromagnetic brain sources. For this purpose gERP and gERMF data were recorded simultaneously using a BTI Magnes 3600 WH (Biomagnetic Technologies, Inc., San Diego) whole-head system with 249 magnetometer and 32 electrodes. Separate ERP and ERMF averaged waveforms were derived time-locked to the onset of the taste stimuli. Finally source analysis was carried out using multi-modal neuroimaging software Curry 6.0.14. The source analysis for the early time range revealed activity in the left and right anterior and mid part of the insula, where in the later time range the sources were located more in the posterior part of the insula and overlaying operculum. This seems conciliate the results previously obtained with fMRI-techniques and the ones obtained with MEG/EEG-based techniques.

Plenary lecture Monday 25 June

Mating switches olfactory coding and preference

Rickard Ignell and The IC-E3 program

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Host plant choice is decisive for insects, but factors that underlie preference are poorly understood. We show that a noctuid moth, the cotton leafworm *Spodoptera littoralis* switches its olfactory preference in response to mating. Following mating, female attraction switches from floral to green leaf odors. Conversely, male attraction switches from pheromone, and floral and green leaf odors to floral odors only. These behavioral changes are mirrored by changes in physiological sensitivity of the olfactory system. Aeration extracts of floral and green leaf volatiles as well as pheromone glands, and selected bioactive compounds in these extracts, elicit state-dependent responses in the peripheral olfactory system, the antennae. In addition, state-dependent changes in sensitivity to these volatiles are observed in the primary olfactory centers of the brain, the antennal lobes. We further show that the modulation in sensitivity correlates with a mating-dependent shift in expression levels of odorant receptors. Functional classification of these receptors is ongoing. Comprehensive biochemical analyses, encompassing juvenile hormone, ecdysteroids, biogenic amines and neuropeptides, reveal dopamine as a key modulator for the mating switch in female moths. This neuroamine induces changes in physiology and behavior of unmated females similar to that observed in mated females. In males, we have evidence that another modulator regulates the behavioral and physiological switch. Studies of olfactory modulation and changes in value-based decisions contribute to the question of how insects acquire new odor templates of ecological relevance, and how new hosts and food sources are acquired.

Symposium 4 "Olfactory and taste circuits" Sunday 24 June

Molecular and neuronal mechanisms for experience-dependent gustatory behavior in *Caenorhabditis elegans*.

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Navigation behavior depending on chemical senses is important for optimizing the chance of survival, especially in invertebrates. In the nematode *Caenorhabditis elegans*, salts were previously considered fixed chemoattractants. However, we recently found salt chemotaxis is finely tuned and highly plastic. When well-fed animals are placed on a concentration gradient of sodium chloride, they are attracted to the salt concentration at which they were previously raised. On the other hand, starved animals avoid the salt concentration under which they were starved. These observations suggest that *C. elegans* can sense and memorize the concentration of sodium chloride, and they have the capacity of associative learning to determine the behavior by associating the salt concentration with availability of food.

Among chemosensory neurons, a single gustatory neuron ASER was found to be able to support cultivation concentration-dependent salt chemotaxis. We therefore monitored the response of ASER sensory neuron by calcium imaging using a genetically encoded calcium indicator and found that ASER always responded to decrease of salt concentration but the magnitude of the response changes depending on the salt concentration of previous cultivation. A pair of downstream interneurons that promote turning behavior responded in an almost all-or-none manner by responding well only after high-salt cultivation. Activity of the Gq/Diacylclycerol/PKC pathway in ASER was found to determine the preferred salt concentration.

On the other hand, avoidance behavior after starvation appears to rely on multiple sensory neurons. The switch of behavior by starvation is mediated by the insulin/PI 3-kinase pathway and calsyntenin homologue CASY-1, both of which act in ASER. Some of the insulin-secreting neurons were found to respond to the absence of food. These results suggest that the two signaling pathways acting in ASER play central roles in the plasticity of chemotaxis behaviors.

Poster session II Poster #204

Artificial odor map based on molecular parameters using the odor separating-measurement system

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Summary: In this sutdy, we focused on an appropriate classification of different odorants like biological odor clustering and constructed the odor map by sensor technology to achieve the odor discrimination. An odor separating-measurement system that extract molecular information of odorants as biological olfaction was developed. With some adsorbents, this system enables high detection and discrimination of odor by obtaining molecular parameters of odorants. Finally, we constructed the artificial odor map, which can classify qualities by their odor-cluster attributes.

Introduction: Mammals form the odor cluster map in the olfactory bulbs (OBs) based on activated by olfactory receptors. Therefore, it is said that odor measurement can be realized by constructing the odor map composed of some clusters, e.g., fatty acids belong to cluster A. Hence, we developed the odor separating system to construct the artificial odor map classified according to each cluster. In the system some adsorbent materials with different properties are adhered on micro-ceramic heaters. By desorbing process by heaters, MOX sensors measure separated odorants. We estimated molecular size and polarity of odorants by the system applied carbon molecular sieves and GC adsorbent materials.

Results: We compared between activity patterns of odor maps and molecular structures of odorants referring the image features from 362 odor maps formed in the rat OBs. Then, we constructed the image map based on molecular parameters. Furthermore, to improve the odor separating system, we developed and embedded nano-filter adsorbents with a specific binding site for odorants in the system. Clusters can be determined by adsorption of odorants to these adsorbents because of a molecular sieve effect of each cluster. Finally, we constructed the artificial odor map using molecular parameters measured by the system. Consequently, the system can quantitatively evaluate various odorants using odor cluster maps.

Poster session I Poster #383

Functional diversity of bitter taste receptor TAS2R16 in primates to natural ligands

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In mammals, bitter taste is mediated by *TAS2R*s, which belong to the large family of seven transmembrane G protein-coupled receptors. Since TAS2Rs are directly involved in the interaction between mammals and their dietary sources, it is likely that these genes evolved to reflect species' specific diets during mammalian evolution. Here, we investigated the sensitivities of TAS2R16s of various primates by using a cultured cell expression system in combination with behavioral tests. We found that the sensitivity of each primate species varied according to the ligand. Especially, the sensitivity of TAS2R16 of Japanese macaques to salicin, a bitter compound contained in the bark of Salicaceae (willow) plants, was much lower than that of human TAS2R16, which was supported by behavioral tests. Japanese macaques ingest the bark of willow trees, containing salicin, while there is no report of such behavior in other species. These results suggest the possibility that bitter taste sensitivities evolved independently by replacing specific amino acid residues of TAS2Rs in different primate species to adapt to accessible food items they use. The amino acid residues responsible for the specificities of each ligands would be discussed in the presentation.

Poster session I Poster #173

Wiring specificity of mitral/tufted cells in the mouse olfactory bulb

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In the mouse olfactory system, odorants are detected by ~1,000 types of olfactory sensory neurons, each expressing a single type of odorant receptor. Olfactory sensory neurons expressing a given type of odorant receptor converge their axons to a pair of glomeruli in stereotyped locations in the olfactory bulb. In each glomerulus, olfactory sensory neuron inputs are relayed to 20-50 second-order neurons, mitral and tufted (M/T) cells. It is known that sister M/T cells innervating the same glomerulus are electrically coupled through the primary dendrites. However, it is not fully understood how the excitatory inputs are modulated by inhibitory circuits. It is also unclear how the inputs to a single glomerulus is represented by 20-50 sister M/T cells. To study the wiring specificity within the olfactory bulb circuits, we used genetic and non-genetic methods to label sister mitral/tufted cells innervating a given glomerulus. We also developed a novel optical clearing reagent, which allowed us to describe almost complete wiring diagram within the mouse olfactory bulb using whole-mount samples. We found that somatic positions as well as the pattern of lateral dendrites are diverse even among sister M/T cells, suggesting that each M/T cells receive and send differential odor information within the olfactory bulb. We are also investigating molecular mechanisms of dendrite targeting and synapse formation/elimination of M/T cells using RNAi screening in vivo.

Poster session II Poster #174

Dual roles of OSN-derived Sema3F in the olfactory circuit formation

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In the mouse olfactory system, olfactory sensory neurons (OSNs) project their axons to specific glomeruli in the olfactory bulb (OB), and creating an olfactory map. Second-order neurons, mitral/tufted (M/T) cells, innervate their dendrites to specific glomeruli and send their axons to the olfactory cortex, transferring the olfactory map information from the OB to higher brain centers. Surgical and genetic studies have demonstrated that axon-derived guidance molecules alone could organize a coarse olfactory map by axon-axon interactions of OSNs. Although the map topography is established independently from the target cues, the map needs to be properly connected with second-order neuron, M/T cells, to make the olfactory circuit functional. How are the primary neuron axons and secondary neurons properly aligned for specific synapse formation between them? Here, we report that OSN-specific knockout of Semaphorin-3F (Sema3F) disrupts both axonal projection of OSNs and migration of mitral cells to the ventral region of the OB. It was found that Nrp2-positive M/T cells are guided to the Sema3F-negative ventral region in the OB, while Nrp2-negative M/T cells stay in the embryonic OB, a prospective dorsal region in the adult OB. These results suggest a novel strategy for axon wiring and synapse formation in the mouse olfactory system: a common guidance molecule (Sema3F) secreted by early-arriving dorsal OSN axons, guides both late-arriving primary neuron (OSN) axons and their partner secondary neurons (M/T cells) to the ventral region of the OB for their proper alignment and synapse formation. Loss of function experiments of Nrp2 revealed that Nrp2-Sema3F signaling also regulates axonal projection of Nrp2-positive mitral cells that target to the medial amygdala. Repeated use of the same pair of guidance molecules may be a general mechanism for the neural circuit formation in the brain.

Poster session I Poster #365

Age-related differences in taste preference in rats.

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Tastes play important roles in detecting nutrients and irritant materials in food. Preference for food tastes is critical for ingestive behavior because higher palatability evokes larger food intake. Animals generally prefer sweetness but not bitterness. Such taste preferences are believed to be affected by aging which causes shift in the dietary and energy requirements. However, the mechanisms of alteration in taste preference by aging still remain unclear. Therefore, to elucidate how aging changes taste preference, we measured the intake of taste solutions using a 48-h two bottle test. We used juvenile (3-6 weeks), young-adult (8-11 weeks), adult (17-20 weeks), and middle-aged (34-37 weeks) Sprague-Dawley male rats. All rats were fed ad libitum during all tests. Taste solutions were NaCl (0.1, 0.3 M), HCl (0.01, 0.1 M), sucrose (0.3, 0.5 M), saccharin (5 mM), quinine (3×10-4, 3×10-5 M), and monosodium glutamate (MSG, 0.1 M). The preference ratios for the 0.5 M sucrose and 0.1 M MSG in the middle-aged group were lower than those in the juvenile and young-adult groups ($p < 0.05$). On the other hand, the preference ratio for the 3×10-5 M quinine in the middle-aged group was higher than those in the juvenile and young-adult groups ($p < 0.05$). There were no significant differences in the preference for HCl and NaCl among groups. Sweet and Umami tastes signal existence of sugar and protein. Thus, it is possible that the decrease in the intake of sucrose and MSG solutions means low energy requirements in aged animals. The higher week old rats showed larger preference for quinine which is not related to a signal of energy. These results indicate that aging caused changes in taste preference of bitter taste solution. Therefore, it is suggested that the aging affects not only homeostasis but also taste preferences.

Symposium 4 "Olfactory and taste circuits" Sunday 24 June

Dynamic odor representations in the olfactory bulb revealed by chronic imaging in awake mice.

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The activity of ensembles of olfactory bulb mitral cells reflects the initial coding of odor representations in the mammalian brain. However, whether mitral cell odor representations are stable or dynamic over long timescales (days, weeks) is unclear. To address this question, we used chronic two-photon calcium imaging in awake mice to study mitral cell activity over months of odor experience. We find that odor familiarization by brief repeated exposure leads to a marked reduction of odor-evoked mitral cell activity without affecting sensory neuron input to the bulb. This mitral cell plasticity is odor-specific; individual mitral cells show a shift in their odor tuning to preferentially represent less-experienced odors. Experience-induced changes in odor representations recover gradually over many weeks and can be repeated with different odors. Thus, odor tuning is dynamically modified by recent experience such that unfamiliar odors strongly activate mitral cell ensembles. Ultimately, this experience-dependent plasticity provides a potential mechanism to encode novel stimuli with greater salience.

Poster session I Poster #385

Intrinsic signal form tongue with gustatory stimulation

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Electro-olfactogram is an established method to record olfactory response directly from the sensory organ. In contrast, there is not a method to record gustatory response from the taste buds or tongue. Especially in human subjects, physiological recording of gustatory response is limited in cortical origin. For developing the method to record gustatory response directly from a tongue, possibility of the application of intrinsic signal recording was examined. Intrinsic signal recording is one of the optical recording methods for neuronal tissue with use of monochrome light without dyes. A device of photo-transistor coupling with an infra red LED (PT-LED) was used for the present experiment. Tongue was illuminated by an infrared LED of 940 nm wave length and the reflection was detected by a photo-transistor. Electric signal from the photo-transistor was amplified and saved on a personal computer via a 14 bit analog-to-digital converter. When a PT-LED was contacted on fungiform papillae and taste solution (e.g. 2% quinine chloride) was administrated, reflection of light intensity was increased but was not increased when the water was administrated. In contrast, there was no optical response was recorded when a PT-LED was located on filiform papillae. Thus, gustatory response from tongue was recorded by optical method. Because intrinsic signal expressed as hemodynamic and absorption of 940nm wavelength infrared ray is decreased when the neuronal tissue is exciting, optical signal from the tongue is seemed the local response of gustatory organ. The present technique is seemed to be a new method to record local gustatory response from tongue.

Poster session II Poster #384

Expression analysis of taste signal transduction molecules in fungiform and circumvallate papillae of *Macaca mulatta*

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In many cases, molecular mechanisms of the gustatory systems have been examined by using rodents as mammalian model organisms. Here we examined mRNA expression of molecules involved in taste signal transduction in fungiform papillae (FuP) and circumvallate papillae (CvP) of macaque, *Macaca mulatta*, by *in situ* hybridization. *TAS1R1*, *TAS1R2, TAS2Rs, and PKD1L3* were exclusively expressed in different subsets of taste receptor cells (TRCs) in FuP and CvP. This finding suggests that TRCs sensing different basic taste modalities are mutually segregated in macaque taste buds. Individual *TAS2Rs* exhibited a variety of expression patterns in terms of the expression levels and the number of TRCs expressing these genes, as in the case of human *TAS2Rs*. *GNAT3*, but not *GNA14*, was expressed in TRCs of FuP, whereas *GNA14* was expressed in a small population of TRCs of CvP, which were distinct from *GNAT3*- or *TAS1R2*-positive TRCs. These results demonstrate similarities and differences between primates and rodents in expression profiles of genes involved in taste signal transduction.

Poster session II Poster #386

Umami solutions affect histaminergic activity via the vagus nerve

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Recently, umami substances, such as monosodium L-glutamate, have been suggested to express various physiological functions via central nervous system. From the functional MRI study using rats, it is indicated that some hypothalamic areas including medial preoptic area and dorsomedial hypothalamus are specifically activated by the intragastric infusion of monosodium L-glutamate solution. The histaminergic system has their cell bodies exclusively in the tuberomammillary nucleus in the posterior hypothalamus, and their fibers are found in almost all the brain regions. The histaminergic system has been suggested to control various physiological functions including feeding behavior, sleep and wakefulness. We previously revealed that taste stimulation can modulate histaminergic tone, and the areas which are activated by L-glutamate are densly innervated by the histaminergic neurons. Thus, we studied the effect of umami solution on histamine release in medial preoptic area in anesthetized rats using in vivo microdialysis. The intraoral stimulation of 0.06M monosodium L-glutamate or 0.1M monopotassium L-glutamate (1ml/min, 2mins) did not alter histamine release. By contrast, histamine release was significantly decreased by the intragastric administration of both of these umami solutions (1ml/min/kg, 10mins). Since both of the solutions showed similar effect on the histaminergic activity, L-glutamate affects hypothalamic histamine release. Effects of umami solutions on histamine release were abolished in vagotomized rats while sham rats exhibited similar decrease of histamine by the intragastric administration of both of the solutions. These findings indicate that L-glutamate acts on glutamate receptors in the stomach and influences histaminergic activity in medial preoptic area via the vagus nerve. Parts of this study were supported by Society for Research on Umami Taste.

Poster session II Poster #92

Physiological properties of antennal-lobe neurons projecting in the mediolateral antenno-protocerebral tract and their GABA immunoreactivities.

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In the moth brain, olfactory information is mediated mainly via three tracts −the medial, the mediolateral, and the lateral antenno-protocerebral tract − projecting in parallel from the primary olfactory center (the antennal lobe) to higher integration areas in the protocerebrum. The major targets of these pathways are the calyces of the mushroom bodies, a region assumed to be important for learning and memory, and the lateral protocerebrum, a premotoric area of the insect brain. Several studies have reported that a prominent portion of the neurons projecting in the mediolateral tract are GABAergic. It is also known that the mediolateral tract neurons omit the calyces by projecting directly to the premotoric region in the lateral protocerebrum, whereas most of the medial and lateral tract neurons target both regions. As regards physiological characteristics of the various projection neurons, a considerable amount of data dealing with response properties of the uniglomerular type passing in the prominent medial tract has been achieved. However, corresponding knowledge about the antennal-lobe out-put neurons projecting in the two remaining tracts, the mediolateral and the lateral, is not yet obtained. Due to this lack of data the function of the parallel pathways is yet poorly understood.

Based on the intracellular recording and staining technique, we present a few multiglomerular projection neurons passing in the mediolateral antenno-protocerebral tract of the Heliothis virescens female. We here present the response profiles of these neurons on which we also have performed GABA immuno-labelling. The double-stained brains have been scanned in a confocal microscope and analyzed by visualization software programs. Possible functions of the mediolateral antenno protocerebral pathway are discussed in accordance with the obtained data.

Contributed talks IV "Olfactory receptors, ligand interactions and transduction mechanisms" Monday 25 June

Olfaction in a moth: antennal transcriptome and unexpected behavior

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Nocturnal insects such as moths are good models to decipher the mechanisms of olfaction since these organisms mainly use their sense of smell to understand their environment and communicate with conspecifics. In addition, since herbivorous moths include many devastating agricultural pest species, a better understanding of the olfactory mechanisms could lead to the identification of original targets to perturb this sensory modality and the insects' impact. We have developed a transcriptomic approach on both adult and caterpillar antennae to identify candidate genes involved in pheromone/host-plant odour detection in the noctuid *Spodoptera littoralis*. Both Sanger and next generation sequencing strategies allowed us to annotate 13685 genes expressed in chemosensory organs. Among them, we described 45 candidate chemosensory receptors and 12 ionotropic receptors, as well as large repertoires of odorant-binding and chemosensory proteins. Comparison between adults and larvae revealed different but somewhat overlapping expression of these chemosensory genes. Surprisingly, we identified pheromone-binding protein expression in larvae antennae, these proteins being characterised in sex pheromone reception and thus referred to as adult specific. This led us to investigate the pheromone-driven behavior in caterpillars, revealing that larvae are attracted toward the adult sex pheromone. A choice test demonstrated that larvae are more attracted to a food source containing pheromone than to food alone and we thus propose that caterpillars use this singular cue to enhance food detection.

This study not only establishes the use of transcriptomic sequencing for the identification of chemosensory receptors in a species for which no genomic data are available, but also paves the way to the identification of unexpected behaviors. In addition, such a chemosensory transcriptome can be used as a reference to further investigate chemosensory modulation via digital gene-expression profiling.

Poster session I Poster #103

Molecular and cellular pathways of NaCl perception in *C. elegans*

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The nematode C. elegans is attracted to 0.1 to 200 mM NaCl and avoids higher NaCl concentrations. Attraction to NaCl is mainly mediated by one pair of sensory neurons, ASE, and involves the guanylate cyclases GCY-14 and GCY-22, the cyclic nucleotide gated (CNG) channel TAX-2/TAX-4 and calcineurin TAX-6/CNB-1. We have identified four new genes involved in attraction to NaCl and show that C. elegans uses two genetic pathways for NaCl taste. One pathway involves tax-2, tax-4 and tax-6. The second pathway consists of tax-2, the CNG channel subunit cng-3, the TRPV channel subunit osm-9 and the Gα subunit odr-3. Avoidance of high NaCl concentrations is mediated by the ASH nociceptive neurons and involves osm-9 and odr-3.

In addition to the above described naïve responses, C. elegans also shows NaCl-taste related learning behavior: after prolonged exposure to NaCl, in the absence of food, the animals avoid all concentrations of NaCl, called gustatory plasticity. To unravel what determines the balance between NaCl attraction and avoidance, and how this balance can be modulated we used Ca2+ imaging. These experiments showed that the ASE neurons of naïve animals respond to both low and high NaCl concentrations, while the ASH neurons only respond to high NaCl concentrations. Interestingly, prolonged exposure to NaCl desensitizes the ASE neurons and sensitizes the ASH neurons. We found that serotonin is involved in both desensitization of ASE and sensitization of ASH. Sensitization of ASH also requires signals from ASE.

Our results suggest that naïve C. elegans are attracted to NaCl, predominantly mediated by ASE, but that this

attraction is overruled by osmotic avoidance, mediated by ASH. Pre-exposure to 100 mM NaCl in the absence of food, sensed by ASE and other neurons, changes this circuit, mediated by serotonin, resulting in desensitization of attraction and sensitization of avoidance.

Poster session II Poster #32

Zap - unzipped: biogenic amine release in the brain of the honeybee in response to an electric shock

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Measuring transient changes in neuromodulators of the brain has long been a challenge in the study of neuroplasticity. In invertebrates, technical limitations have meant that attention has focused primarily on long-term and often global changes in brain amine levels. However, in the context of learning (classical conditioning), detailed information is required about transient changes occurring within specific brain regions. For this, measurements with high spatial and temporal resolution are crucial. For example, although dopamine has been shown to be necessary and sufficient for the pairing of a conditioned stimulus with mild electric shock, it remains unclear whether dopamine alone is released in response to this punishment, in which brain region(s) it is released, the kinetics of its release and how release is affected by stimulus intensity.

Fast scan cyclic voltammetry (FSCV) allows neurotransmitter release to be detected at very high sensitivity and at a sub- second temporal level. While this technique has long been used to detect biogenic amine release in vertebrates, FSCV has not been widely applied in insects. However, miniaturization of the electrodes now allows well-defined brain regions to be probed while limiting neural structure damage, even in tiny insect brains.

We have taken advantage of the FSCV technology to measure the release in vivo of biogenic amines in the brain of the honey bee in response to mild electric shock. Here we characterize the relationship between amine release and the intensity/duration of electric shock stimuli, quantify changes in amine release over subsequent stimulations and examine the effects of neuropharmacological manipulation of dopamine release on aversive learning in the bee.

Symposium 14 "Higher olfactory processing - Delwart Symposium" Tuesday 26 June

Pheromone processing in a sexually dimorphic olfactory circuit

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We are investigating the neural circuit basis of olfactory perception in Drosophila. Olfactory information enters the fly brain in 50 glomeruli of the antennal lobe. Second order neurons then project to two structures, the mushroom body, which is required for olfactory learning but apparently dispensable for innate olfactory behaviour, and the lateral horn. We have previously demonstrated that olfactory input to the lateral horn is spatially stereotyped across individuals and that pheromone and general odours are mapped to different zones. We hypothesise that this poorly characterised brain centre is where odour information starts to be transformed into behaviourally relevant representations.

One initial focus has been on the processing of the pheromone signal cVA, a male pheromone that is repulsive for other males but a female aphrodisiac. We have recently shown striking anatomical dimorphisms in the dendrites of third order lateral horn neurons that appear to receive cVA information from incoming projection neuron axons. Two small groups of neurons show male specific overlap while a third shows overlap in females. We are currently investigating the physiology of these neurons in both sexes; we hypothesise they will be the first neurons to show differential pheromone responses between the sexes.

cVA is repulsive for males. What do they find stimulating? Volatile fly pheromones of female origin have been long

sought but remain elusive. However Richard Benton's group (Lausanne) has recently demonstrated that a specific food derived compound, PAA, can act as a male aphrodisiac. Intriguingly, we have shown in collaboration that unlike other food signals PAA information projects to the pheromone processing centre of the lateral horn. It is therefore likely that third order neurons in this area integrate information about food and fly odours that regulate fly courtship.

Symposium 23 "Evolution of chemosensory systems " Wednesday 27 June

Widespread pseudogenization of the sweet taste receptor gene Tas1r2 in the order Carnivora

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Much of mammalian sweet taste is mediated by the taste receptor T1R2 + T1R3, a heteromer that recognizes a wide variety of sweet-tasting compounds, including natural sugars, non-caloric sweeteners and certain macromolecular compounds, including some proteins. We showed previously that the well-known behavioral indifference of cats toward sweet tasting compounds can be explained by the pseudogenization of the *Tas1r2* gene which encodes the T1R2 receptor. We reasoned that other exclusively meat-eating species might also have an inactive form of this gene. To test this hypothesis, we sequenced the entire coding region of *Tas1r2* from twelve carnivore species some of which were exclusively meat eaters whereas others were more omnivorous or even herbivorous. We found that the pseudogenization of *Tas1r2* is widespread among those species that are exclusively meat eaters. Of the 12 species tested, a defective *Tas1r2* was found in the banded linsang, fossa, spotted hyena, sea lion, fur seal, pacific harbor seal, and Asian small- clawed otter. None of the mutations that disrupt the open-reading frame are shared among these carnivorous species, with the exception of closely-related fur seal and sea lion. Fittingly, the selective pressure is markedly reduced in carnivore species with a pseudogenized *Tas1r2*. We behaviorally tested two of the genotyped species. Consistent with their *Tas1r2* genotypes, the Asian otter (defective *Tas1r2*) showed no preference for sweet tasting compounds, including both natural and non-caloric sweeteners while the spectacled bear (intact *Tas1r2*) preferred sugars and some non-caloric sweeteners,. Taken together, our results indicate that the loss of a functional *Tas1r2* is surprisingly widespread among meat eating carnivores probably due to the relaxation of selective pressures maintaining receptor integrity.

This work was made possible by NIH DC010842 to P.J. and institutional funds from the Monell Chemical Senses Center.

Poster session I Poster #283

Does chemosensory exposure to propionic acid impair error processing in a visual task? Results from an event related potential study

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Chemosensory stimulation has repeatedly been assumed to interfere with cognitive performance but results have been inconsistent due to varying cognitive tasks, use of substances with varying trigeminal potency, or differently sensitive individuals. In the working environment such background stimulation often occurs due to handling volatile organic compounds. In a previous study, we demonstrated reduced behavioral accuracy in human volunteers performing a motor-inhibition task during exposure to 10 ppm propionic acid (PA). We now investigated this effect on the neuronal level using event-related potentials (ERPs). We focused on ERP components representing error processing due to their sensitivity to affective contexts and the strong relation of chemosensory stimuli to emotions.

Methods: 24 volunteers were whole-body exposed to PA at concentrations: 0.3 (odor control), 5 (variable from 0-10) and 10 ppm (occupational exposure limit). Reaction times and accuracy were recorded in the motor-inhibition task three times during each 4-h exposure. In a subgroup of six subjects, error components were analyzed in response-locked ERPs.

Results: Response accuracy in the ERP subgroup showed a trend for impaired accuracy with increasing PA exposure in trials including motor inhibition (Inhibition*Concentration: $p = .059$, repeated measures ANOVA). These results reflected findings from the whole study group. The error-related positivity (Pe) was reduced in grand averages of both high exposures as compared to the odor control condition, indicating a modification of later, conscious error processing steps. This result did not reach statistical significance, probably due to the small group size.

Conclusions: (1) ERP measurements offer a valuable possibility to examine effects of chemical exposures at the neuronal level. (2) Replication of our findings in larger study groups is needed.

Conference participation was supported by ECRO travel grant.

Poster session II Poster #132

Maternally inherited peptides function as strain identity chemosignals in mice

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In rodents, each individual has a great complex of chemosignals produced by the animal and acquired from the environment and from conspecifics. The resulting chemical signatures are complex and variable mixtures that are still poorly understood. The main olfactory system is specialized to sense volatile molecules, whereas the vomeronasal system is specialized to pump in relatively non-volatile chemosensory cues following direct contact. Peptide ligands of MHC class I molecules and major urinary proteins have been suggested to be chemosignals that are required to signal individual identity. We hypothesized that three mitochondrially-encoded peptides, NADH dehydrogenase 1 (ND1), NADH dehydrogenase 2 (ND2) and cytochrome c oxidase 1 (CO1), can be utilized to convey strain-specific information among individuals. Because ND1, ND2 and CO1 are encoded in the mitochondrial genome, they are maternally inherited, formylated at the N-terminal methionine at the time of synthesis and polymorphic among inbred mouse strains. ND1 is a nine-amino-acid peptide derived from the N-terminus of NADH dehydrogenase subunit 1: formyl (f)-MFFINXLTL. The identity of the sixth amino acid varies among different strains and can be either A, I, T, or V. Similarly, ND2 and CO1 have respective sequences of fMNPITLXII where the seventh position is A or T and fMFXNRWLFS where the third position is I or T. To test this hypothesis, we used the strain-dependent pregnancy block paradigm and studied the effect of formylated and nonformylated variants of ND1, ND2 and CO1. When single synthetic peptide variants of a different strain type were added to urine from BALB/c males, they differentially increased its effectiveness in blocking pregnancy following mating with a BALB/c male. However, the addition of BALB/c-specific peptides was ineffective. These results suggest that mitochondrially encoded peptides are capable of conveying strain identity in the pregnancy block effect.

Poster session II Poster #284

The nasal cycle: functional neural asymmetry reflected in the nose

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Nasal airflow is asymmetric, the nostril with greater flow alternating over time. This *nasal cycle* (NC) reflects lateralized autonomic nervous system innervation: sympathetic causing vasoconstriction and increased airflow, parasympathetic causing vasodilatation and decreased airflow. Here we set out to characterize the temporal dynamics of the NC, and test the hypothesis that the NC is causally related to hemispheric functional brain asymmetry. Diurnal patterns of NC were measured using a novel battery-powered belt-worn device that allows 24-hour continuous recording of airflow in each

nostril. Diurnal patterns of 19 healthy subjects (10F) revealed large variability between and within subjects. Although all subjects cycled, cycle length ranged from 15 min to 7.5 hours (mean=1.8 hours). Cycling differed in sleep and wake, by number of cycles (sleep= 1.33±1.36 wake= 7.83±2.71, $t(6)= 7.17$, $p<0.0005$), cycle length (sleep= 2.01±0.84, wake=4.87±2.25, $t(6)=3.45$, $p<0.05$), and inter-nostril correlation (sleep=-0.54±0.49, wake=0.05±0.34, $t(6)=2.72$, $p<0.05$). To test the hypothesis that functional brain asymmetry may drive the NC, we presented participants with either left-hemisphere-dependent verbal tasks, or right-hemisphere-dependent spatial tasks. Strikingly, tasks drove NC such that nasal flow increased in the left nostril following the verbal task, and in the right nostril following the spatial task (R/R+L slope, Verbal=-0.19±0.07, Spatial=0.05±0.07, $F(1,11)=6.33$, $p<0.03$). An EEG pilot in 5 subjects confirmed that these tasks indeed drove the expected hemispheric imbalance ($t(4)=3.23$, $p<0.05$). To test the hypothesis that NC may drive functional brain asymmetry, we measured EEG following forced unilateral nasal breathing. This manipulation failed to drive EEG asymmetry in the 5 subjects tested to date. We will continue to investigate this path with the intention of ultimately driving activity in injured hemispheres (stroke/trauma/etc) through manipulations of nasal airflow.

Poster session I Poster #285

Effect of different flavors on frontal cortex blood flow and respiratory cardiovascular systems after soup intake with nutritional equivalence.

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This research examined the effect of two soups with different flavor and nutritional equivalence on the palatability and the physiological parameters (the frontal cortex blood flow and the respiratory cardiovascular system). The significant difference in palatability between two soups was confirmed by sensory evaluation. As for the physiological parameters, the significant differences between two soups were seen in the responses of the oxygenated hemoglobin at the right side of the frontal region, the heart rate, and the respiratory quotient after soup intake. These differences may be explained as a series of responses, which include the rise of oxygenated hemoglobin at the right side of the frontal region by low palatable soup, followed by the sustained rise of the heart rate in cardiovascular system and the depression of the respiratory quotient in respiratory system as stress responses.

Poster session I Poster #131

Goofy: a novel Golgi membrane protein with specific expression and crucial function in the olfactory sensory neurons

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Transmembrane and secreted proteins play important roles in a variety of developmental and functional aspects of olfactory sensory neurons. By using the signal sequence trap method, we discovered novel molecule *Goofy* from mouse olfactory epithelium cDNA library. *Goofy* gene encodes a type I integral membrane protein with no significant sequence homology with any other proteins. *Goofy* mRNA is specifically and abundantly expressed in the olfactory and vomeronasal sensory neurons. The developmental onset of *Goofy* mRNA expression is much earlier than that of olfactory marker protein (OMP) expression. Goofy protein is predominantly localized to the Golgi apparatus in both immature and mature olfactory sensory neurons. To elucidate Goofy function, we generated *Goofy*-deficient mice and analyzed their biochemical, anatomical, physiological, and behavioral phenotypes. The *Goofy*-deficient mice displayed abnormal localization of adenylyl cyclase III, shortening olfactory cilia, and reduced electrophysiological and behavioral responses to odorants.These results suggest that Goofy plays a crucial role in proper functioning of the olfactory sensory neurons.

Poster session I Poster #433

Biochemical and immunocytochemical characterization of olfactory signaling molecules in the non-olfactory system

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Olfactory sense is mediated by specialized olfactory receptor neurons (ORNs) in the nose. Olfactory signaling molecules, such as G-protein (G_{olf}) and Adenylyl Cyclase 3 (AC3), have recently been reported to be found outside of the olfactory system, suggesting that the olfactory sense may play a role in other tissues. However, ectopic expressions of olfactory signaling molecules (G_{olf}, AC3, OMP, ORs) and their functional roles still remain to be elucidated. This study demonstrates the presence of olfactory signaling molecules in other tissues by systemically using a double-antibody immunoisolation/immunodetection procedure, western blotting, and RT-PCR analysis. In addition, immunohistochemical analysis was utilized to find OMP positive cells in the stomach, bladder, testis, kidney, heart, lung, liver, thymus, thyroid, duodenum, and spleen. Unexpectedly, OMP positive cells were found in some tissues through immunohistochemical analysis. Gene expression of olfactory receptors was also observed in other tissues. The gene expressions of OMP, G_{olf} and AC3 in several tissues were noticeable in a unique cell type. Among them, tissues related to the gastrointestinal (GI) tract and urinary system showed high expression of olfactory signaling molecules only in restricted regions, which was unexpected. These results suggest that olfactory signaling molecules are extensively present in certain regions of tissues and the chemical sensing through the molecules may be related to food digestion, absorption, excretion, and other functions.

Poster session II Poster #402

Fgf signaling controls pharyngeal taste bud formation through miR-200 and Delta-Notch activity.

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Taste buds, the taste sensory organs are conserved in vertebrates and composed of distinct cell types including taste receptor, basal/presynaptic and support cells. Here we first characterize zebrafish taste bud development and show that compromised Fgf signaling in the larva results in taste bud reduction and disorganisation. We determine that Fgf activity is required within pharyngeal endoderm for formation of Calb2b+ cells and reveal miR-200 and Delta-Notch signaling as key factors in this process. miR-200 knocking down shows that miR-200 activity is required for taste bud and in particular Calb2b+ cell formation. Compromised *delta* activity in *mib-/-* dramatically reduces the number of Calb2b+ cells and increases 5HT+ cells. Conversely, larvae with increased Notch activity and *ascl1a-/-* mutants, are devoid of 5HT+ cells but have maintained and increased Calb2b+ cells, respectively. These results show that Delta-Notch signaling is required for intact taste bud organ formation. Consistent with this, Notch activity restores Calb2b+ cell formation in pharyngeal endoderm with compromised Fgf signaling but not after miR-200 knock-down. Altogether this study provides genetic evidence supporting a novel model where Fgf regulates Delta-Notch signaling and subsequently *miR-200* activity to promote taste bud cell type differentiation (1).

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Poster session I Poster #323

Paracetamol masking bitter taste prodrugs- a computational approach

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Bitter taste is a major problem in the food and medicine industries. As several oral pharmaceuticals and bulking agents have unpleasant, bitter-tasting components, pediatric patients resist taking these medicines due to their bitterness, leading to lack of patient compliance.

Drugs interact with taste receptor on the tongue to give bitter, sweet or other taste sensation. Altering the ability of the drug to interact with bitter taste receptors could reduce or eliminate its bitterness. This can be achieved by an appropriate modification of the structure and the size of a bitter compound.

Paracetamol is an odorless crystalline compound with a bitter taste widely used as pain killer and to reduce the temperature of patients with fever. Its prodrug, phenacetin, on the other hand, lacks or has a very slight bitter taste. Acetanilide, a compound similar to paracetamol but lacking the phenolic hydroxyl group, has a burning taste and lacks the bitter taste characteristic for paracetamol. These combined facts suggest that the presence of hydroxyl group on the *para* position is the major contributor for the bitter taste of paracetamol. Hence, it is expected that blocking the hydroxyl group in paracetamol with a suitable linker could inhibit or inactivate the interaction of paracetamol with its bitter taste receptors and hence mask its bitterness.

We will discuss the mechanism for certain reactions of enzyme models having the potential to be good carriers to bitter taste drugs such as paracetamol and others having hydroxyl group. The prodrugs consist of drugs connected by their hydroxyl groups with the linkers. They are expected not to have bitter taste due to lack of ability to be engaged in a hydrogen bonding with the bitter taste receptors. In addition, those prodrugs might release the parental drugs in a controlled manner upon their degradation when exposed to physiologic environments.

Poster session I Poster #35

Context-dependent flower odor preference in the hawk moth *Manduca sexta*

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Recognition and location of a suitable host plant or a nectar-rich flower is a complex task for the olfactory system of herbivorous insects. Plants and their flowers emit characteristic blends of volatile compounds that have to be identified by the insects. Adult tobacco hornworms (*Manduca sexta*) have been shown to use specific flower odor blends to pinpoint nectar sources. Our hypothesis was that also non-flower, "green" odors of the plant play in concert with flower odors during nectar source location.

We performed two-choice experiments in a wind tunnel with 3 days old, unmated and starved females to odour sources that emitted exclusively flower blends, exclusively "green" plant blends or a mixture of both. We used two different host plants (*Datura wrightii*, *Nicotiana attenuata*) and a non-host plant (*Brassica oleracea var. gemmifera*). When testing flower or plant blends of a single plant species versus a combination of both, moths displayed a strong preference of the combined blend over the single blends, suggesting an attraction synergy between flower and plant blends. However, we did not observe this kind of interaction when we combined flower and plant blends of different species, suggesting that the synergy between plant and flower blends occurs within species, only. In conclusion, our results suggest that foraging moths pinpoint flowers not only by the flower-emitted blend, but in addition pay attention to the blend of the complete plant. Interestingly the moths seem to have an innate template telling them whether the detected flower blend fits to the detected plant background.

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Poster session II Poster #126

Effect of milk fermented with lactic acid bacteria and yeast on the preference of rats

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Lactic acid bacteria (LAB) have been widely used in the manufacture of fermented milk because of their flavor and health benefits. In Japan, "CALPIS", a unique traditional milk product prepared by fermentation with LAB and yeast, has a long history in the marketplace and is well known and loved for its unique flavor. The unique flavor of "CALPIS" is generated by the natural process of LAB and yeast during the course of fermentation in two stages. Many studies have focused on the health benefits of fermented milk, however, little is known about the preference for fermented milk. The objective of the present study was to investigate the role of LAB and yeast on the preference for fermented milk products, using a rat model.

Test samples of fermented milk were prepared by cultivation of skim milk with a starter containing *Lactobacillus helveticus* with *Saccharomyces cerevisiae* (LY-FM) or without *S. cerevisiae* (L-FM). A non-fermented milk product was also prepared as a control sample (N-FM). The sugar contents and acidities of L-FM and N-FM were adjusted to the same value of LY-FM by the addition of sucrose and lactic acid. A comparative evaluation of the test samples was conducted using two-bottle preference tests on rats.

Rats showed significant preference for LY-FM compared to N-FM and L-FM. In contrast, there was no significant difference in preference between N-FM and L-FM. To understand the influence of aroma compounds, especially those originated by yeast in LY-FM, anosmic rats were prepared by intranasal administration of zinc sulfate. The preference for LY-FM was significantly reduced in the anosmic rats. These results suggest aroma compounds, originated by *S.*

Poster session I Poster #325

Importance of Umami-Taste Sensation. Part 1: Development of Umami Sensitivity-Testing Method Using Filter Paper Disc

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Measurement of recognition threshold (RT) for four basic tastes (sweet, salty, sour, and bitter tastes), using filter paper disc (FPD) method is clinically used for the assessment of taste disorder in Japan. FPD is generally applied to three different innervated areas (bilateral: anterior tongue / posterior tongue / soft palate). However, recognition of umami taste has not been developed as a FPD method and analysis of umami-taste disorder has yet to be conclusively reported. Therefore we tried to develop an FPD method for umami sensitivity with solutions of a typical umami substance, monosodium glutamate (MSG). We measured RT of healthy young-adult subjects (n=50, 21.0 ± 4.2 yr) and healthy elderly subjects (n=22, 80.2 ± 7.9 yr) using six-level of MSG test solutions of 1, 5, 10, 50, 100 and 200 mM. There was no significant difference between young-adult and elderly subjects for the region threshold. In both subject groups, approximately 80% subjects showed RT of 5 or 10 mM for the posterior tongue and the soft palate, whereas 10 and 50 mM for the anterior tongue. We then tested the influence of instruction of umami taste on RT using 50 healthy young-adult subjects. The instruction was performed by tasting small amount of 200mM MSG solution just before the test. There was no significant difference in RT with and without the instruction in either subject. In conclusion, we have established umami sensitivity test using FPD and the method could be applied for the assessment of umami-taste disorder because it has high convergence of normal RT both in young and elderly subjects and because the RT is not influenced by the instruction of umami. We also found that anterior tongue has significantly higher sensitivity for umami than the posterior tongue and the soft palate.

Poster session II Poster #326

Quantitative analysis for the hedonic effects of sweet tastants in the bitter masking.

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Bitter taste is primitively aversive, but its aversiveness is attenuated by addtion of sweet tastants. We feel it as masking effects to bitter taste, though there are no direct interaction between bitter and sweet tastants. On behavior tests for taste evalutaion with mice, lick numbers to the test solutions depend on their favorability. So, we analyzed the favorability of various concentration of bitter solution and evaluated EC50 by brief access tests. We also evaluated EC50 of the bitter test solution with sweet compounds and calcurated masking effects to bitter taste from amount of the EC50 change. Same procedure was conducted by the mice injected an opioid antagonist to investigate the hedonic effects of sweet compounds to the masking effects. As a result, the addition of 2.5 mM sodium saccharin showed 1.6 fold increase in the EC50 of both denatonium benzoate and quinine-HCl solutions. This result suggest that the additon of 2.5 mM sodium saccharin would suppress aversiveness of bitter taste to approx. 40%. Naloxon injection (3 mg/kg IP) could not diminish the masking effects by sodium saccharin.

Poster session I Poster #391

Bitter reception in experimental rats with low zinc

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It is known that low zinc is one of reasons to cause taste disorder. Previous histochemical studies showed that low zinc causes atrophy of taste cells and delay in turnover of taste cells. In the present study, we examined the behavioral changes in response to bitter stimulation in the experimental animals with low zinc. In addition, the expression of c-fos in the neurons within parabrachial nucleus (PBN), one of the important relay nuclei of gustatory transmission, following application of bitter stimuli as well as expression of α-gustducin, a molecule related to bitter reception, in the taste buds of experimental animals with low zinc. Experimental animals were created by feeding low zinc diet for 4 weeks from postnatal day 21. Those animals showed the low level of zince in serum. Number of cells within single taste bud, and percentage of cells showing α-gustducin per single taste buds of the experimental animals were almost identical to those of normal animals. Behavioral anaysis by two-bottle preference test showed that animals with low zinc preferred 0.01mM quinine compared with normal animals. Following application of bitter stimuli (0.001M quinine) to the posterior portion of tongue, c-fos neurons in PBN significantly increases in number in normal animals. In low zinc animals, however, the number of c-fos neurons in PBN was not changed following bitter stimulation. The present results indicate that low zinc affects the perception of bitter substance and transmission of bitter information from periphery to central, and there is no apparent histological changes in peripehral receptors.

Contributed talks V "Human olfaction" Monday 25 June

Human odorant receptors and odor quality perception

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Humans sense odors with a combination of over 400 odorant receptors (ORs). Each odor binds to a combination of ORs and it is known that perceptually similar odors often bind to similar combinations of receptors. It is therefore plausible that the perceived quality of an odor depends at least partially on the combination of ORs it activates. This can be studied in human subjects because OR genes in humans are unusually variable and for most ORs it is therefore possible to find subjects that do not carry a functional variant of it.

To test if the combination of ORs with which an odor is sensed influences how the odor quality is perceived we psychophysically tested in 400 subjects the perception of perceptually similar odors, like the two odorous steroids androstenone and androstadienone. The subjects selected verbal descriptors that describe the perceived odor quality. They were also tested for their ability to discriminate the odors and binary mixtures in which the two odors were mixed at different ratios.

There are three human ORs that are sensitive to both androstenone and androstadienone and one OR that is sensitive to androstadienone but not to androstenone. Our hypothesis is that for subjects that lack the OR that is selectively activated by androstadienone the two odors smell more similar and are therefore more difficult to discriminate. This hypothesis can be tested by sequencing the ORs sensitive to the odorous steroids to determine if there is an effect of genotype on the ability to discriminate androstenone and androstadienone.

In addition to the two odorous steroids we also tested (+)-carvone and (-)-carvone, two enantiomers whose smell is described as caraway and spearmint, and isovaleric acid and isobutyric acid, two structurally and perceptually similar carboxylic acids. The goal of these experiments is to find general principles how the combination of ORs with which an odor is sensed influences the odor's perceived smell.

Poster session II Poster #130

Peripubertal exposure to male odor influences both female puberty and adult sexual preference in mice

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In mice, olfactory signals emitted by males regulate female reproductive function: for example, peripubertal exposure to male urinary odors accelerates female puberty onset (the so called Vandenbergh effect). Even if the effect of male odors on female physiology at puberty has been extensively studied, whether peripubertal exposure to male odors also influences female's behavior during adulthood is at present unclear. Here we show that a peripubertal exposure to male bedding odors induced both acceleration of puberty onset in females and earlier occurrence of preference for volatiles in male odor. Females exposed to castrated male bedding odors did not show puberty acceleration but show an early preference for male odors. Females exposed to clean bedding showed neither puberty onset acceleration nor an early preference for male odors. Finally, at a more advanced age where all females expressed a preference for volatile male odors, females exposed to intact male bedding odors peripubertally showed a higher preference in comparison to females stimulated with castrated male or clean beddings. Comparative chemical analysis using solid phase microextraction coupled with gas chromatography and mass spectrometry of beddings from intact males and castrated males revealed

around ten volatile organic compounds that where shared across treatments while the 2-sec-butyl-4,5-dihydrothiazole appeared as specific to intact male bedding. Some compounds, such as the 3,4-dehydro-exo-brevicomin, were however present at a lower concentration in castrated male bedding than in intact male bedding. As a whole, the dissociation observed between puberty onset acceleration and olfactory sexual preference suggest that both effects rely on a qualitative and/or quantitative difference between male (intact vs castrated) bedding odors, and strongly suggest the involvement of 2-sec-butyl-4,5-dihydrothiazole and 3,4-dehydro-exo-brevicomin, known to be testosterone dependent.

Poster session II Poster #366

Differential effects of experience with Na-cyclamate on human taste sensitivity to high-intensity sweeteners

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Experience with Na-cyclamate (Na-c) significantly increases human taste sensitivity for glucose, fructose, and maltose, but not sucrose (Gonzalez et al., 2007, 2008; Collins et al., 2010). Human psychophysical, hamster chorda tympani nerve, and *Drosophila melanogaster* receptor cell action potential data suggest mechanisms in the peripheral nervous system (Faurion et al., 2002; Hassan et al., 2006; Gonzalez et al., 2009). We have suggested that binding of the treatment compound with the receptor molecule (T1R3 in humans) leads to changes in binding or other steps in the receptor response to the test compound. Here we tested whether Na-c treatment leads to a similar increase in taste sensitivity to Na-c, sucralose, and D-tryptophan (D-tryp). Na-c is a salt of cyclamic acid that binds T1R3. Sucralose is sucrose modified with two Cl atoms, while D-tryp is an amino acid. Modeling and molecular data suggest that sucralose and D-tryp bind the Venus flytraps (VFT) of T1R2 and T1R3 (Morini et al., 2005; Zhang et al., 2008). Participants rinsed their tongues with 4 mM Na-c or water for 10 sec once a day for 10 days. On day 11 or 12, they tasted an isosweet concentration series of Na-c, sucralose, or D-tryp, each concentration paired with water, and indicated which of each pair was "the sweetener." Participants treated with Na-c showed increased sensitivity to Na-c ($p=0.03$) and sucralose ($p=0.004$) and decreased sensitivity to D-tryp ($p=0.006$). These results indicate that the mechanism(s) for experience-induced changes affect(s) stimulation by various sweeteners differently. Although sucrose and sucralose both bind the VFT(Zhang et al., 2008), the differing results for these sweeteners may suggest different receptor mechanisms. Differential effects of allosteric modulators (Servant et al., 2010) and behavioral and neurophysiological data from flies (Higgins and Kennedy, 2001) also suggest mechanisms for sucrose and sucralose. Supported by NIH NIDCD R15DC009042 to LMK.

Contributed talks III "Mixed session" Monday 25 June

Mycbp2, an E3 ubiquitin ligase at the crossroads of axon guidance, synaptogenesis and mental retardation

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MYCBP2 is a strongly conserved E3 ubiquitin ligase that regulates axon and synapse development through interactions with multiple signalling pathways. Mycbp2 binds to and negatively regulates the tumour suppressor gene tuberin (TSC2) and its downstream mTOR kinase signaling pathway. TCS2 inactivation is responsible for the human neurodevelopment disorder tuberous sclerosis, a syndrome associated with mental retardation and autism. In order to better understand the role of Mycbp2 in axon guidance and synaptic formation we examined the development of the olfactory system in several mutant lines of Mycbp2 mice. In all lines we observed a similar and very significant loss of innervation phenotype in a large dorsal domain in the olfactory bulb. Since Robo2 expressing axons normally innervate the dorsal bulb we examined the topography of these axons during development of the olfactory nerve pathway. Ventrally we observed an ectopic population of primary olfactory axons expressing Robo2 which failed to target the dorsal bulb. Next we crossed heterozygous Robo2 +/- mice against heterozygous Mycbp2 +/- mice. Each single heterozygous line of mouse has

normal wild-type innervation of the olfactory bulb by primary olfactory axons. However, double heterozygotes exhibit an axon guidance phenotype in the olfactory bulb similar to, although not as severe, as that observed in the Mycbp2 -/- homozygote mice. These results have revealed that Mycbp2 is genetically interacting with Robo2 and that both genes are acting in the same pathway. These results have provided the first evidence that axon guidance molecules lie downstream of Mycbp2 and that their altered expression can contribute to Mycbp2 phenotypes associated with neural circuit formation in the mammalian nervous system.

Contributed talks V "Human olfaction" Monday 25 June

General olfactory sensitivity: candidate genes and their genomic variations

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Genetic variations in olfactory receptors are believed to contribute significantly to the reported diversity in odorant-specific phenotypes, such as detection threshold and quality perception. We ask what genes whose variations could similarly explain general olfactory sensitivity (GOS) and its extreme case, congenital general anosmia (CGA). We thus aim to investigate how genetic factors cause some individuals to generally detect odorants better or worse than others. Our working hypothesis is that genetic variations in olfactory auxiliary genes, including those mediating olfactory signal transduction and those involved in olfactory sensory neuron development and integrity may constitute the genetic basis for general phenotypic variability. We performed a literature survey, seeking functional *in vitro* studies, mouse gene knockout studies or human disorders with olfactory phenotype. We also mined published high throughput data of olfactory sensory neurons gene expression, and in parallel performed our own next-generation transcriptome sequencing in human and mouse olfactory epithelium and bulb, so as to identify sensory-enriched transcripts. This entire process led to the generation of a prioritized list of ~420 annotated olfactory auxiliary genes, identified by 10 different data sources, 180 of these genes are yet unannotated in the context of olfaction. Based on public genome variation repositories, we identified human gene variants – single nucleotide polymorphisms, insertion-deletions and copy number variations that affect the olfactory auxiliary genes. This compendium of genes and their variations should assist in rationalizing the great inter-individual variation in human overall olfactory sensitivity, including in next-generation whole-exome sequencing of ~20 CGA individuals.

Poster session II Poster #294

Transfer of terpene-related odorants into human milk - a process involving dietary, environmental and pharmacological considerations

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Transfer of odorants from the maternal diet into human milk has become the subject of scientific interest in terms of the possible influence on the immediate milk flavor profiles, as well as on food preferences in later life due to these early flavor experiences.

Our research aims at characterizing potential odorant transfer on a molecular basis, both sensorially and analytically. We have primarily focused our studies on monoterpenes, since these odorants are ingredients in many herbal foods, and are also present in other common sources such as cosmetics and cleaning agents, as well as being associated with environmental factors.

In step one of the present study, 100 mg 1,8-cineole were taken by breastfeeding mothers in form of the orally administered pharmaceutical Soledum®. The second part of the study focused on concentrations within the range of a

normal diet; here, herbal tea (fennel-anis-caraway infusion) was ingested by the mothers and the subsequent appearance of the tea constituents limonene, 1,8-cineole, fenchone, estragol, carvone, trans-anethole, anisaldehyde and anisketone was monitored in breast milk. Additionally, blank milk samples were analyzed to reflect the normal diet and environmental conditions.

A change in flavor of the human milk was observed only in relation to the high dose of 1,8-cineole. Analytical quantification verified this finding, with concentrations of 1,8-cineole up to the mg/kg range. By comparison, concentrations of the monoterpenes associated with herbal tea intake were mostly in the lower µg/kg to ng/kg range.

Additional findings were the presence of large inter- and intra-individual differences, which were most likely due to individual absorption and transfer processes. Moreover, the experiments showed that in relation to the general background concentrations of the monitored monoterpenes in breast milk, additional intake of low doses could not elicit any further odorant increase in the milk.

Poster session I Poster #327

Narrow band imaging (NBI) is useful for lingual papilla examination in patients with taste dysfunction

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Narrow band imaging (NBI) is a novel optical technique that enhances the diagnostic capability of the gastrointestinal and otorhinolaryngological endoscopes by illuminating the intraepithelial papillary capillary loop using narrow bandwidth filters in a red-green-blue sequential illumination system. NBI is helpful for finding tumor lesion in its early stage. Here we report that NBI is also useful for lingual papilla examination in patients with taste dysfunction. NBI system emits two specific wavelength waves (415 nm and 540 nm), which are hemoglobin absorption band and enhance to obtain fine images of the superficial microvascular architecture. We studied the lingual papillae of subjects with and without taste dysfunction using flexible otorhinolaryngological fiberscope with NBI. We compared NBI to conventional white-light imaging. Capillaries in lingual papillae contrasted better with surrounding tissue in NBI than in white-light imaging, making fungiform papillae involving blood flow easy to count. Taste-dysfunction sufferers' tongues showed decreased lingual papilla blood flow. However, NBI examination detected normal microvascularity within lingual papilla in patients with subjective taste dysfunction caused by the flavor dysfunction or psychological diseases. The count of fungiform papillae involving blood flow was correlated with the results of conventional electric taste examination. In conclusion, we found NBI to be handy and useful in clinically diagnosing taste dysfunction.

Poster session II Poster #300

Brain structural and cognitive basis of OI deficits in Alzheimer`s disease (AD)

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Objectives: The anatomical and cognitive substrate underlying the deficits in odor identification (OI) abilities in neurodegeneration remain undetermined. The main goal of this study was to explore the relationship between OI function and the volumetric and cognitive measures in healthy elderly compared to patients with amnestic MCI (aMCI) and Alzheimer`s disease (AD) of mild degree, to better understand the neurobiological basis of OI preservation or loss in AD.

Material and Methods: 18 patients were recruited from the Memory Clinic, Department of Geriatrics, St.Olavs University Hospital in Trondheim, 12 patients with a diagnosis of aMCI and six with AD of mild degree. 30 age-matched controls were also included. All patients and controls underwent psychophysical measurements (odor identification and taste) and a structural MRI scan. In addition, they were evaluated with a set of cognitive tests (MMSE, verbal memory and delayed recall ,Ten Words Test).

Results: Both odor identification tests (B-SIT, SSIT) and the taste test showed significant differences between patients and controls ($p < 0.0005$). Brain volumes significantly reduced in patients were the total of Hippocampus, as well as the left and right Hippocampus, and the Amygdala ($p<0.0005$).The total Ventricular volume was significantly increased in the patients ($p<0.0005$). In the combined group of patients and controls both B-SIT and SSIT scores were significantly correlated with MMSE, as well as verbal memory and delayed recall (p 0.01). MMSE, Verbal memory and delayed recall were significant correlated with Total Hippocampus, Left and Right Hippocampus and Amygdala brain volumes. Taken together, central structures in MTL are important for OI abilities, and cognitive abilities like verbal memory seems to affect performance on tasks measuring OI abilities.

Poster session II Poster #36

A conserved non-pheromone olfactory receptor in *Manduca sexta*

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An extensive set of members of olfactory gene families was recently reported for the tobacco hornworm *Manduca sexta*, a model species in olfactory research (Grosse-Wilde et al, 2011).As is common to insects in general, odorant receptor (OR) coding genes showed remarkable sequence diversity and low conservation both within the species and in comparison to other Lepidoptera. However, besides already known conserved subgroups like the pheromone receptors, MsexOR-31 was revealed as equally highly conserved, with homologs only in other lepidopteran species as e.g. *Bombyx mori* and *Heliothis virescens.* We speculate that the comparatively high degree of conservation between these OR genes indicates their involvement in an as-of-yet unknown behaviour of importance. Here we present an extensive analysis of MsexOR-31 in *Manduca*, using the "empty neuron" system of *Drosophila melanogaster* as heterologous expression system to functionally characterize MsexOR-31. This project was funded by the Max Planck Society.

Poster session I Poster #129

The role of early experience on L-felinine detection thresholds in house mouse.

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Felinine is a unique sulfur-containing amino acid found in the urine of domestic cats and select members of the Felidae family. In our early studies we showed that exposures of mice *Mus musculus* to urine from feral cats *Felis catus* under semi-natural conditions significantly affected survivorship of offspring. Manipulations with the diet of predator and non-predator urine donors revealed the key role of sulfur-containing compounds. In more recent study we examined the influence of the precursor of potential Felidae family pheromone L- felinine on reproductive output in mice. We recorded number of newborn pups, sex ratio, weight of pups at weaning. Exposure to L-felinine affected litter size ($n=40$, $p<0.05$) and sex ratio in mice ($n=40$, $p<0.001$) in favour of males. By the day of weaning in control groups of animals average weight of pups was significantly ($p<0.001$) higher then in the exposed to L-felinine. Also we observed long lasting elevation of corticosterone under L-felinine exposure ($n= 10$, $p<0.001$). Our data indicated that L-felinine could play a role of a potential heterospecific chemical signal. Taking this into account in the current study we investigated the role of early experience with predator scents on the detection thresholds of the compound in adult house mice. Newborn pups were exposed to cotton balls soaked with L-felinine (0.05% w/v, 0.05 ml, US Biologicals) directly in their home cages over period of two weeks after eyes open. Period for odor exposure was selected based on our previous studies on sensitization to odors in house mice (Voznessenskaya, Wysocki, 1994; Voznessenskaya et al., 1999). Control group of pups was exposed to tap water. Behavioral thresholds were estimated using olfactometer (Knosys, USA).

Detection thresholds ranged from dilution 1:3,000 to 1: 15,000. Early experience with L-felinine significantly altered detection thresholds in adult mice of 3-4 months of age. Supported by RFBR 10-04-01599 to VVV

Poster session I Poster #395

Preference for saltiness, food neophobia, and vegetable intake

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We explored relationships between preference for saltiness in foods, food neophobia, and vegetable intake among parents of young children. Derived from an ongoing family study in Southwest Finland (the STEPS study), the present data included 767 women (18-44 years old, mean 30.8 years) and 610 men (17-56 years old, mean 32.8 years), covering all education levels. We analyzed the self-reported vegetable intake (portions consumed per week; portion defined as, e.g., 1 tomato or 2 small carrots), the preferred saltiness in foods (2 questions about experienced saltiness in convenience food and meals eaten in restaurants etc. compared to meals cooked at home), and food neophobia (the Food Neophobia Scale, FNS; range 10-70) using validated questionnaires. For food neophobia, we classified the participants as "low", "average", and "high" (scores 10-19, 20-35, and 36-70, respectively), and for salt preference as "low" and "high". Women had lower preference for saltiness than did men (Chi^2, $p<0.001$), but no difference existed in food neophobia. In contrast, high education level was associated with both low preference for saltiness and low food neophobia ($p<0.001$). Preference for saltiness was not associated with vegetable intake after gender was considered in the analysis. Instead, women used more vegetables than did men (means 15 vs. 11 portions per week), participants with low neophobia more than those with high neophobia (15 vs. 9), and participants at high education level more than those at low education level (15 vs. 10) (3-way ANOVA, main effects: food neophobia $p<0.0001$, education level $p=0.0007$, gender $p<0.0001$; no interactions). Age did not correlate with vegetable intake or FNS score. Taken together, present results suggest that food neophobia, together with gender and education, but not preference for saltiness, are associated with vegetable intake in parents of young children. This work was supported by the Academy of Finland (MS252005, MS256176, HL121569).

Symposium 18 "Olfactory neuroethology" Tuesday 26 June

Spatial representation of odorant valence in an insect brain

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Brains have to decide whether and how to respond to detected stimuli based on complex sensory input. The vinegar fly *Drosophila melanogaster* evaluates food sources based on olfactory cues. Here we performed a behavioural screen using the vinegar fly and established the innate valence of 110 odorants. Our analysis of neuronal activation patterns evoked by attractive and aversive odorants suggests that even though the identity of odorants is coded by the set of activated receptors, the main representation of odorant valence is formed at the output level of the antennal lobe. The topographic clustering within the antennal lobe of valence-specific output neurons resembles a corresponding domain in the olfactory bulb of mice. The basal anatomical structure of the olfactory circuit between insects and vertebrates is known to be similar; our study suggests that the representation of odorant valence is as well.

Poster session I Poster #367

Identification of neural projection between the primary gustatory area and the thalamus, using diffusion tensor imaging

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Location of the primary gustatory area (PGA) in human beings has been the issue of argument for a long time since Penfield and Boldrey (1937). Although intensive electrophysiological experiments on gustatory areas have been made on subhuman primates, research on human beings had been limited to clinical observation of patients with brain damage as in Motta (1959). Recent development of imaging techniques has yielded various non-invasive methods, e.g., functional magnetic resonance imaging (fMRI), positron emission tomography (PET) and magnetoencephalography (MEG), and allows us to measure the cerebral activities of living human subjects without surgical invasion. PET and fMRI have revealed activation at the frontal operculum or the superior insula anterior to the central sulcus and suggested that the PGA presents at the same region in humans as in subhuman primates. MEG, however, located the PGA at the transition between the parietal operculum and insula because this area was the fastest activated bilaterally in most subjects after gustatory stimulation of the tongue tip, followed by the frontal operculum. There, currently, exists discrepancy for cortical location of the human PGA among neuro-imaging methods. In order to dissolve this discrepancy, we investigated neural projection between cortical candidates of the PGA and the thalamus. We investigated neural projection between the thalamus and the transition area between the parietal operculum and insula, and that between the thalamus and the frontal operculum, or anterior insula using whole-brain diffusion tensor imaging (DTI) analysis, as PGA should have efferent projection from the thalamus. This technique found neural connection between the parietal op. and thalamus, but no connection between the frontal op and thalamus.

Poster session I Poster #301

Blockade of interleukin-6 receptor suppresses inflammatory reaction and ameliorates functional recovery following olfactory system injury

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We previously reported that recovery in the olfactory system depends on severity of local injury and anti-inflammatory treatment with steroid is effective in improving recovery outcome after olfactory nerve transection. Clinically, however, steroid administration is not recommended in an acute phase of head injury because of no evidence of its efficacy in head injury and concern about its side effects as hypertension and infection. Recently, it is reported that interleukin-6 (IL-6) plays an important role in inflammatory reaction and blockade of IL-6 receptor suppresses inflammatory reaction. Actually, anti-IL-6 receptor antibody (IL-6R-Ab) is used for clinical treatment of refractory inflammation as the rheumatoid arthritis. The present study was designed to investigate if IL-6R-Ab is also useful for functional recovery in the olfactory system following injury. We made a model of severe injury by performing olfactory nerve transection using a rigid stainless steel blade in transgenic (OMP-tau-lacZ) mice. Anti-mouse IL-6R-Ab (MR16-1) was injected intraperitoneally just after the nerve transection. Histological assessment of recovery within the olfactory bulb was made at 5, 14, 42 and 70 days after injury. X-gal staining was used to label the degenerating and regenerating olfactory nerve fibers and immunohistochemical staining to detect the presence of reactive astrocytes and macrophages. MR16-1-injected animals showed significant smaller areas of injury-associated tissue, less astrocytes and macrophages, and an increase in regenerating nerve fibers in a dose-dependent manner. Behavioral study using avoidance conditioning and electrophysiological study showed better functional recovery of olfactory system in MR16-1-injected mice than in control animals. These findings suggest that IL-6R-Ab can be useful as a therapeutic drug for olfactory dysfunction by head injury.

Poster session II Poster #286

Influence of implicit information-manipulation of the same odor stimulus -using affective priming method (2nd report)

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In this study, we investigated whether "implicit" odor information-manipulation is effective in affecting participants' subjective evaluation to the odor, controlling their conscious cognitive biases. We have previously obtained results showing that individuals presented subliminal "disgust" faces (the negative group) perceived the odor as less intensive as compared with those presented "happiness" faces (the positive group) only in female participants (ECRO 2010). Thus we selectively took data from female participants in this study, while the experimental procedure taken was mostly the same as that reported in ECRO 2010. The odor used as a target stimulus was anise seed oil, unfamiliar odor to the Japanese people. As priming visual stimuli, pictures of faces with "happiness" expression were presented in the positive group, while those with "disgust" expression were presented in the negative group. The priming stimulus was presented for 20 msec together with the target odor stimulus. Although 20 msec is not short enough to be "subliminal", we adapted the duration because our previous finding suggests that the duration of 20 msec carries effects of both implicit and explicit features. We further assigned the participants another task, in which they were checked whether they could actually "see" the facial expression with a forced choice procedure and divided them into either the "implicit" or the "explicit" group. Results showed that values on odor pleasantness evaluation "converged" depending on the information-manipulation in the "implicit" group, whereas those values "spread" over wide range in the "explicit" group presumably due to each participant's conscious cognitive biases. These results suggest that "implicit" odor information-manipulation controls or minimizes various conscious cognitive biases that would mask or disturb the effects of targeted specific cognitive effects that we manipulate as an independent variable.

Poster session I Poster #111

Odor map in the zebrafish olfactory bulb-2: GCaMP Ca^{2+} imaging

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Fish can detect a variety of odorants in water, which evoke fundamental olfactory behaviors important for their survival, such as searching for foods, finding mates, and escaping from danger. The odor information is initially represented in the olfactory bulb (OB) by patterns of neural activation on the array of glomeruli. Thereby, individual odorants activate specific sets of glomeruli in the OB. However, functional significance of the spatial odor representation in the OB is not fully understood with regard to the translation of odor inputs into behavioral responses. To genetically dissect olfactory neural circuits responsible for these olfactory behaviors, we have successfully used enhancer/gene trap strategy in combination with Gal4/UAS system in zebrafish. In this study, we expressed genetically encoded calcium indicator GCaMP-HS (or GCaMP4), improved versions of GCaMP under the control of the Gal4/UAS system, and measured odorant-evoked neural activities in the OB of adult zebrafish. In transgenic lines with GCaMP expression in the olfactory sensory neurons, we detected significant increase of calcium signals in distinct glomeruli of the OB upon application of defined odorants or putative pheromones, such as amino acids (feeding cue), bile acids (social cue), prostaglandin F2α (sex pheromone), and conspecific skin extract (alarm pheromone). Further experiments are now in progress to visualize the neural activities in the OB upon stimulation with a wide range of odorants and to create the functional and comprehensive odor map underlying different olfactory behaviors in zebrafish.

Poster session I Poster #287

Hormonal dependence of chemosensory perception

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Purpose: Considering gender differences, studies showed greater olfactory performance, including higher odor sensitivity and better odor identification in females than in males (Larsson et al., 2004; Doty & Cameron, 2009). This female advantage seems to be due to hormonal factors (Russell et al., 1980; Evans et al., 1995) or derive from variables associated with these hormonal changes. Previous studies demonstrated a significant impact of cycle phase on olfactory thresholds in females. In this study we investigated the impact of cycle phase and oral contraceptive intake by assessing a broad range of olfactory performance, i.e. identification, discrimination and threshold as well as hedonic valence and intensity. Materials/ Methods: Eighty healthy subjects (60 f, 18-44 ys) were included. The female group consisted of 20 pill users and 40 females without hormonal intake who were further divided into follicular and luteal phase. Olfactory performance of all participants was assessed twice using the "Sniffin' Sticks" battery and intensity and pleasantness ratings of n-butanol were collected. Females who had their first test during follicular phase were retested in their luteal phase and vice versa. Results: Data analysis revealed significant gender differences in odor discrimination and odor identification with females outperforming males. Moreover, more sensitive odor thresholds and lower pleasantness ratings emerged in the luteal phase. Notably, a significant positive correlation between duration of oral contraception and olfactory performance emerged pointing to better performance with longer intake. A better overall performance was found at the second testing. Only females who were testes first in the follicular phase showed a significant improvement. Conclusion: The results of the presented study indicate that odor performance is affected by menstrual cycle phase and oral contraception and thus indicate that this ability is modulated by hormonal changes.

Poster session II Poster #128

Immune responses and neuroepithelial degeneration induced by intranasal administration of Poly(I:C) in the mouse olfactory mucosa

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We investigated the morphological changes and innate immune responses in the mouse olfactory mucosa induced by intranasal administration of a synthetic double-stranded (ds) RNA, polyinosinic-polycytidylic acid [Poly(I:C)], a molecular mimic of replicating virus. Mice received three intranasal administrations of Poly(I:C) (50μg each) or normal saline every 24 hours. The olfactory mucosae were fixed at various intervals after the first administration (8 hours, 3, 9 and 24 days) and immunohistochemically examined regarding the time course and extent of 1) neuroepithelial degeneration and regeneration, 2) infiltration of inflammatory cells, and 3) expression of the molecular signaling via Toll-like receptor 3 (TLR3), the receptor for innate immune response to dsRNA. Apoptosis of the olfactory receptor neurons had already begun at 8 hours. The expression of phosphorylated NF-κB, a downstream signal of TLR3, was also upregulated at this time point. The olfactory neuroepithelium degenerated most severely at 9 days and then regenerated almost completely by 24 days. Regarding the inflammatory cell kinetics, neutrophils predominantly infiltrated the olfactory neuroepithelium at 8 hours and exuded into a nasal cavity at 3 days. Macrophages and T lymphocytes also infiltrated at 8 hours in the lesser magnitude and remained in the olfactory mucosa until 24 days. To examine if neutrophil-derived cytotoxic enzymes are involved in the damage of olfactory neuroepithelium, mice were pretreated with neutrophil elastase inhibitor (Sivelestat) before the administration of Poly(I:C). This pretreatment significantly suppressed the neuroepithelial degeneration of the olfactory mucosa by Poly(I:C). These findings suggest that innate immune responses via TLR3 and subsequent release of elastase by neutrophils may play an important role in the pathogenesis of postviral olfactory disorder.

Poster session II Poster #432

Enhancement of preference for dried-bonito dashi (a traditional Japanese fish stock) by prior food experience

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The dried-bonito *dashi* is a traditional Japanese fish stock that improves palatability of various dishes, probably via enhancement of umami taste. We have found previously that preference behavior for *dashi* varies among rodent strains, with the least preference by C57BL/6 strain mice. By using C57BL/6 mice, here we investigated experimental methods how to enhance preference for dried-bonito *dashi* in 48-h two-bottle choice tests. The commercial *dashi* employed were the "*Hondzukuri Ichiban-dashi Katsuo*" (Ajinomoto, Japan) which was 5- to 10-fold dense-taste stock of hot-water extracted dried bonito. As the dry matter components was 4% (w/w) on a weight basis, the commercial *dashi* was considered as 4% solution. In the ascending concentration series, the mice showed a low preference for *dashi*: the most preferred concentrations were observed between 1.2% and 2% solutions with the maximal preference for 65% (just above the water preference levels). In the descending concentration series, however, the maximal preference increased greatly to 95% and the concentration-preference functions shifted to left for 10,000-fold. As a result, the preference was observed in wider concentration ranges (5 log units vs. 1 log unit). Next, we have found that prior experience of *dashi* ingestion (for 10 days) induced a great enhancement of preference for *dashi* even in ascending concentration series. An induction of moderate preference enhancement was observed following experience for monosodium glutamate or inosinate (umami) but NaCl (salty), lactate (sour) and histidine (bitter) had no effects. These results suggested importance of prior experience of *dashi* ingestion for the development of *dashi* preference. Experience for umami substance partly contributes to the enhancement. Experience-based enhancement of preference may involve postingestive consequences associated with ingestion.

Symposium 20 "Aquatic olfaction" Tuesday 26 June

Evolution of olfactory receptor gene repertoires and function

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Most olfactory receptor gene families evolve rapidly, following a birth-and-death mode of evolution. It is widely assumed that the purpose of such dynamic evolution is adaptation to species-specific ecological niches and communication signals. We have phylogenetically characterized the fish *taar* gene family and find this family to be an extreme example of evolutionary dynamics, with several instances of positive selection. At the other extreme lies the V1R-related *ora* gene family, which we found to be highly conserved, with orthologues of individual genes detectable in species as far apart as shark, zebrafish and frog. We have deorphanized receptors from both families. Receptors were found to be narrowly tuned to high affinity ligands, and some of these ligands are able to elicit distinct innate behavior. Preliminary observations are consistent with the hypothesis that the evolutionary dynamic of receptor genes does not correspond to a similar dynamic in biological function.

Poster session II Poster #206

Olfactory receptors are differentially equipped with combinations of evolutionary conserved C-terminal intracellular trafficking signals

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Since the discovery of odorant receptors (OR) about 20 years ago, only as few as 21 human OR could be assigned to specific agonists. The bottleneck in de-orphaning OR is their sub-optimal functional expression at the plasma membrane of hetero-logous cell systems. For many trans-membrane proteins, such as G protein-coupled receptors (GPCR) and ion channels, it is well known, however, that their trafficking to the plasma membrane is controlled by evolutionary conserved, short C-terminal amino acid motifs. By analysing the entire OR repertoires of 8 vertebrate species, and in sharp contrast to earlier publications, we identified 11 evolutionary conserved C-terminal amino-acid motifs, 6 of which had been described to operate as retrograde, and 5 as anterograde trafficking signals in non-olfactory GPCR or ion channels. From 4695 analyzed OR, 76% carry C-terminal trafficking signals, with marked differences between class-I and class-II OR, where 84% and 75% carry trafficking signals, respectively. It has been shown that class-I and class-II OR sensory neurons differ in their axon targeting to the olfactory bulb along an anterior-posterior axis, and that such axon targeting of olfactory sensory neurons is regulated by OR-derived cAMP levels. Hence, a combinatorial and differential equipment of OR with C-terminal trafficking signals, which regulates the number of functionally expressed OR, may be the molecular prerequisite for an instructive role of OR in axon targeting. Moreover, a C-terminal sequence tailored for robust membrane expression in heterologous cell systems of OR may finally enable their functional de-orphaning.

Poster session II Poster #368

Role of different docking sites of TrkB receptor during gustatory development

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The neurotrophins Brain-derived neurotrophic factor (BDNF) and Neurotrophin-4 (NT-4) differentially influence the development of the gustatory system in mice. It has previously been shown that BDNF is important for chemo-attraction and regulation of gustatory neuron targeting during embryonic development. NT-4 and BDNF both bind the TrkB receptor, however, NT-4 regulates gustatory neuron number at an earlier stage than BDNF, and does not influence neuronal targeting. In order to elucidate this mechanism, we are investigating which TrkB intracellular signalling pathways are required for specific aspects of gustatory development by examining *in vivo* point mutations in TrkB docking sites: a) the Shc docking site ($Trkb^{SHC}$), who's recruitment leads mainly to activation of Ras/MAPK pathway as well as PI3K, b) the PLCγ1 docking site ($Trkb^{PLC}$) that generates inositol-1,4,5-trisphosphate and diaglycerol and c) both docking sites ($Trkb^{D}$).

Examining geniculate ganglion survival at different stages of embryonic development, (i) immediately prior to the onset of innervation from this ganglion into the tongue, E12.5, and (ii) just after the neurons reach their targets at E14.5, we found that the Shc adaptor site, and thus signalling pathway/s activated through this site downstream of the TrkB receptor, is involved in regulating survival of the geniculate ganglion neurons, while the PLCγ adaptor site is not involved.

BDNF has also been shown to influence neuronal targeting into the tongue. We therefore are examining the influence of each of the above docking sites on the innervation of neural/taste buds at different developmental stages. Since innervation is not complete until E14.5, we are looking at two later stages, E16.5 and P0. Our data indicate that BDNF, acting via its PLCγ adaptor site, influences neuronal targeting in mice. Together our findings so far suggest that specific aspects of gustatory development depend upon different docking sites of TrkB receptor.

Symposium 11 "The stimulus – odor space and chemometrics" Sunday 24 June

In search of the structure of human olfactory space

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We analyzed the responses of human observers to an ensemble of monomolecular odorants. Each odorant is characterized by a set of 146 perceptual descriptors obtained from a database of odor character profiles. Each odorant is therefore represented by a point in a highly multidimensional sensory space. We studied the arrangement of odorants in this perceptual space. We argue that odorants densely sample a two-dimensional curved surface embedded in the multidimensional sensory space. This surface can account for more than half of the variance of the perceptual data. We also show that only 12% of experimental variance cannot be explained by curved surfaces of substantially small dimensionality (<10). We suggest that these curved manifolds represent the relevant spaces sampled by the human olfactory system, thereby providing surrogates for olfactory sensory space. For the case of 2D approximation, we relate the two parameters on the curved surface to the physico-chemical parameters of odorant molecules. We show that one of the dimensions is related to eigenvalues of molecules' connectivity matrix, while the other is correlated with measures of molecules' polarity. We also show that responses of human observers to mixtures can be fit within the curved manifolds obtained for monomolecular odorants described above. The dimensionality of mixture space, however, exceeds the dimensionality of monomolecular space by about one for the same level of explained variance.

Poster session II Poster #110

Immediate Early Gene egr1 and tyrosine hydroxylase expression in larval and adult zebrafish brain

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The sense of smell plays a crucial role in zebrafish kin recognition which depends on imprinting on the 6th day. Likely, zebrafish larvae use kin recognition for aggregating (shoaling) and adult females for inbreeding avoidance. Here, we investigated the potential role of the immediate early gene *egr1* in odor perception and kin recognition by analyzing *egr1* (*krox-24, zif 268, ngfi-a* and *zenk*) gene expression in imprinted and non-imprinted larvae (3 through 8 days) and in adult imprinted females. We noted extensive basal activity of *egr1* in imprinted/non-imprinted larval and in adult female forebrain and dorsal midbrain, but not in the ventral midbrain and in the hindbrain. Forebrain domains included the olfactory bulb and the secondary olfactory supracommissural nucleus of the ventral telencephalon (Vs), the possible homologue of the medial amygdala.

In mice, *egr1* mediates activity-dependent tyrosine hydroxylase (TH) expression in olfactory bulb dopaminergic periglomerular neurons. Odor deprivation through naris-occlusion revealed almost complete absence of *egr1* and TH expression levels in these dopamine cells, but not in other *egr1* expressing cells. Therefore, double-labeling for TH and *egr1* was performed in brains of adult imprinted female zebrafish in order to check for co-localization for TH and *egr1* in the olfactory bulb and Vs. The results demonstrated that TH/*egr1* double-label is the exception in the zebrafish brain. However, in the olfactory bulb, *egr1*/TH co-localization occurs in periglomerular cells (as in the mouse) and in cells of Vs. Odor deprivation through application of TritonX-100 in adult female zebrafish led to unilateral downregulation of *egr1* and *egr1*/TH positive periglomerular cells in the olfactory bulb suggesting similar olfactory processes in mice and zebrafish.

Poster session I Poster #39

Odorant binding proteins and olfactory receptors of *Anopheles gambiae*:Antennal expression pattern and sensilla co-localization

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In the malaria vector Anopheles gambiae (Ag) female mosquitoes rely on olfaction to find a blood host, sugar sources and oviposition sites, whereas nectar feeding males mainly locate host plant odors. Both genders detect odorants by means of olfactory sensory neurons (OSNs) located in olfactory sensilla on the antennae and the maxillary palps. Upon entering a sensillum odor molecules are supposed to be transferred by odorant binding proteins (OBPs) through the sensillum lymph towards olfactory receptors (ORs) in the dendritic membrane of the OSN. Large gene families encoding diverse AgOBPs and AgORs have been annotated from the A. gambiae genome. While for several AgOBPs and AgORs very high transcript levels in female antennae suggest distinctive roles of the encoded proteins in female olfaction, the number and antennal topography of the expressing cells is largely unknown. We have visualized the cells expressing sex- biased AgOBPs and AgORs in the antenna by using whole mount fluorescent in situ hybridization (WM-FISH). This allowed us to determine their number and distribution and to scrutinize for a sensilla co-localization by determining the relative position of AgOBP and AgOR positive cells. It was found that both sexes express the same AgOBPs and AgORs, but there exists a significant sexual dimorphism concerning the number and topography of the expressing cells. Different AgOBP- as well as AgOR-types differed in the number and antennal distribution of the expressing cells. Two-color WM- FISH experiments revealed co-expression of distinct AgOBPs by the same cells. Moreover, cells expressing certain AgOBPs laid in direct proximity of cells expressing distinct AgORs, indicating a co-localisation in the same sensillum. This may suggest a possible interplay of the AgOBP/AgOR pairs in the detection of odorants. This work was supported by a grant from the European community's Seventh framework Programme project ENAROMaTIC (FP7/2007–2013; agreement FP7-222927).

Poster session II Poster #40

Modulation of olfactory processing in male *Spodoptera littoralis*

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Moths primarily depend on odor stimuli to locate nectar sources, mates and mating substrates. Although we have accrued a general understanding of how moths detect these signals, little is known about how this information is modulated by the physiological status of the insect.

In wind tunnel experiments we showed that virgin male *Spodoptera littoralis* were attracted to female sex pheromone as well as to nectar source and mating substrate signals. Interestingly, three hours after mating males showed no attraction to sex pheromone nor to odors of mating substrate. However, behavioral attraction to floral volatiles was maintained. Twenty-four hours post mating, attraction to sex pheromone as well as mating substrate was almost fully restored.

Through electrophysiological analyses of antennal responses to sex pheromone, floral and mating substrate volatile extracts and their bioactive components we observed that floral-evoked responses were independent of mating status. Conversely, responses to pheromone and mating substrate showed a marked decrease three hours after mating, but were fully restored at 24 hours after mating. Calcium imaging of the antennal lobe revealed a similar change in the olfactory representation of the different volatile extracts and to specific components thereof.

Based on our observations we conclude that mating triggers significant, rapid but short-term changes in the olfactory system of males.These changes are tightly correlated to the observed mating-induced switch in olfactory-guided behavior. Furthermore, our data suggest that food source and mating substrate/pheromone information is modulated through separate pathways, each correlated to the ecological, behavioral, and physiological requirements of the insect.

Poster session I Poster #207

Quantitative imaging of the membrane order and olfactory signalling elements.

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Olfactory receptor neurons (ORNs) in the nasal cavity are highly polarised epithelial cells. ORNs extend dendrites and cilia into to the mucus layer for odour recognition and signal transduction. Ciliary membranes are likely to be enriched in cholesterol and sphingolipids and are hence more ordered than the basolateral membrane of the cellular body. The signal transduction machinery for odour recognition, which is located in ciliary membranes, comprises of a large family of G-coupled receptors and number of ion channels. This therefore poses the question how membrane organisation affects theirs localisation and function. Here, we aim to investigate the structure- function relationship of ORN membranes.

To achieve this, we employ the fluorescent probe Laurdan, which is sensitive to the polarity of local membrane environment. Laurdan exhibits a blue-shift in emission peaks, when localised in ordered membrane domains. As a neuronal model, we chose to use HEK293 cells transfected with either ORI7, a classic G-protein coupled receptor; CNGA2, a cyclic nucleotid activated channel; and ANO2, Ca^{2+}- activated Cl^- channel. Proteins are fused to a fluorescent protein, enabling us to quantify the membrane order of the lipid environment of the respective protein. Further, we will expand our imaging methodology to visualize these proteins in ORN in the intact nasal epithelium of mice infected with adenovirus. This will provide evidence of correlation between membrane organization and localisation of signalling elements within membrane.

Poster session II Poster #208

Expression of thyroid hormone receptor in the salmon olfactory epithelium

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Thyroid hormone plays an important role in the regulating multiple cellular and metabolic processes, including cell proliferation, cell death, and energy metabolism, in various organs and tissues of vertebrates. It is generally accepted that anadromous Pacific salmon (*Oncorhynchus* spp.) imprint some odorants of their natal streams at the downstream migration, and use their olfaction for discriminating those streams during spawning migration. Both the serum thyroid hormone levels and the specific binding values of thyroid hormone in the olfactory epithelium were markedly increased during the downstream migration. However, thyroid hormone receptor (TR) expressions in the olfactory epithelium have not been confirmed in vertebrates. We investigated gene expressions of TR isoform in the chum salmon (*O. keta*) by molecular biological and histochemical techniques. Expressions of TR mRNA were detected in the olfactory epithelium by reverse transcriptase polymerase chain reaction (RT-PCR). The nucleotide sequencing demonstrates the existence of a remarkable homology between the RT-PCR product and a part of the hormone-binding domain of other teleost TR beta isoforms. By in situ hybridization using a digoxygenin-labeled salmon olfactory TR cRNA probe, signals for salmon olfactory TR mRNA were observed preferentially in the perinuclear regions of the immature olfactory receptor cells, as the protein gene product 9.5 (PGP9.5)-immunopositive cells. Our results provide the first detection of TR gene expression in olfactory epithelium, and indicate that TR beta is involved in the cell differentiation and/or the cell maturation of the olfactory receptor cells in Pacific salmon.

Poster session I Poster #209

Variation in the human olfactory subgenome and its impact on olfactory perception

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The olfactory receptors (ORs) are a family of G-Protein coupled receptors that provide the molecular basis for the detection of volatile odorant molecules by the central nervous system. In humans, the *OR* gene family comprises about

400 functional genes and 600 non-functional pseudogenes. Furthermore, the *OR* gene family shows a high degree of genetic variability between individuals. Common single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) lead to specific patterns of functional and non-functional *OR* genes in each individual subject, resulting in "different noses for different people" (Menashe *et al.*, Nat Genet, 2003).

At the same time, it has long been known that people differ in their ability to perceive certain odorants. In the most striking cases, subjects are completely devoid of the ability to perceive certain odorants, although their sense of smell functions normally in general. This phenomenon is known as specific anosmia and has been shown to have a genetic basis in some cases.

We obtained genomic DNA samples from subjects with a specific anosmia for one of several odorants. We then used massive parallel sequencing (MPS) techniques to look for genetic variation in the human *OR* repertoire that could explain the occurrence of specific anosmias. Experiments were performed on pools of DNA samples to maximize throughput and cost-efficiency. Furthermore, we established a novel strategy to align MPS reads unambiguously to the *OR* gene reference sequences, which show a high degree of sequence similarity. Results indicate that most specific anosmias may be complex traits which are caused by the combined effects of several genetic and/or environmental factors.

Acknowledgements: This project was funded by SPP 1392 "Olfaktorik" of the Deutsche Forschungsgemeinschaft (DFG).

Poster session II Poster #102

Roles of diacylglycerol signaling in migration toward preferred salt concentration in *C. elegans*

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Salt is an important taste cue for finding and discriminating food for animals. The soil nematode *C. elegans* has long been thought to chemotax to salt (sodium chloride). However, we have recently found that it is attracted to the salt concentration at which it has been fed. In addition, animals avoid the salt concentration at which they experienced starvation. Therefore, salt chemotaxis of *C. elegans* is based on memory of salt concentration and modulated by food availability associated with the salt concentration. Animals migrate up or down salt gradient by switching the bias of klinokinesis, in which the frequency of turning is modulated by the time derivative of salt concentration. Input from single gustatory neuron ASER is essential and sufficient for chemotaxis to both directions. Up- or down-regulating diacylglycerol (DAG) signaling in ASER promotes migration toward higher and lower concentrations, respectively. *In vivo* DAG monitoring indicated that the DAG level in ASER is elevated by the decreases in environmental salt concentration. AIB interneurons, one of the postsynaptic interneurons of ASER, are known to positively regulate turning behavior. Calcium imaging experiments showed that AIB is activated by the decreases in salt concentration only when stimulus concentration was lower than cultivation concentration. These results suggest that under fed conditions, decreases in environmental salt concentration up-regulate DAG signaling in ASER, which probably sensitizes synaptic transmission from ASER to AIB that evokes turning behavior, and result in migration toward higher concentration.

Poster session I Poster #127

The role of glucocorticoids in reception of sex pheromones in house mouse

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Olfactory cues play an important role in regulation of complex forms of social behavior, including sexual behavior in mammals. A number of studies demonstrated a direct involvement of accessory olfactory system (AOS) in regulation of male sexual behavior in mammalian species. While the role of sex hormones in regulation of perception and analysis of chemical signals are studied very well, the role of stress hormones remains quite unclear. At the same time suppressive effect of stress on reproduction of mammals is a well known issue whereas influence of stress on signal perception in vomeronasal system remains unknown. Our earlier studies showed the suppression of the response to receptive female chemical cues of vomeronasal receptor neurons in males under exposure to emotional or cold stress. Number of Fos-positive cells in VNO receptor epithelium of males in response to receptive female chemical signals was significantly reduced under stress exposure. According to our data after exposure to both types of stress (low temperatures and cat odour) male mice demonstrated no preference towards receptive female odor versus non-receptive while in control group of animals we observed such a preference. These alterations in behavior were accompanied by increased plasma corticosterone. In search of putative mechanism in the current study, we investigated the expression of steroid receptors (glucocorticoid, GCR, androgens,AR, and mineralocorticoid, MCR) in VNO receptor epithelium using immunohistochemical techniqies. We detected a profound GR-immunoreactivity in VNO receptor tissue of male mice but not AR-immunoreactivity or MR-immunoreactivity, whereas it was present in control tissue. Abundant expression of GCRs in VNO receptor tissue suggests possible direct action of stress hormones on receptor cells. The data obtained indicate glucocorticoid involvement in female chemical cues perception in vomeronasal system. Supported by RFBR 10-04-01599 to VVV

Contributed talks I "Modulation of the olfactory system (Linnaeus Symposium)" Monday 25 June

Molecular and neural correlates of circadian neuromodulation on olfactory sensitivity in the periphery of the American cockroach

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Olfaction is an important sensory modality in insects that is essential for host location, finding mates and foods, and other inter and intra-specific interactions in nature. Based on many studies so far, insect olfactory systems in peripheral as well as central nervous systems appear to be highly plastic. Previous studies indicated that both olfactory sensitivity and memories exhibited daily fluctuations that are regulated by the circadian system in cockroaches. Also, a line of recent studies suggested that insect olfactory systems are modulated by both biogenic amines and neuropeptides. However, it remains elusive how these molecules modulate olfactory system in the peripheral systems. In the present study, our aim was to characterize the structure and organization of these signalling systems in the peripheral olfactory system of the American cockroach, *Periplaneta americana*. Our present study utilizing in situ hybridization, qRT-PCR, and electrophysiology coupled with pharmacological and RNA interference approaches indicate that neuropeptide, tachykinin, and its receptors are involved in the regulation of olfactory sensitivity in the antennae of the American cockroach. Here we also show that tachykinin-producing cells also express receptors for the biogenic amine, which causes the neuronal modulation in olfactory perception. These results suggest that both biogenic amine and tachykinin peptide in the antennal neurons are important regulators in the periphery. We propose the hypothesis that biogenic amines could regulate the release of tachykinin from the antennal neurons, which , in turn, modulate the sensitivity of olfactory receptor neurons.

Poster session II Poster #34

Manduca sexta pheromone receptors

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Moth pheromone detection is one of the most well described examples in olfactory research. Moth pheromone communication is typically used to locate mating partners over long distances, a behavior directly related to the fitness of the individual. Recognition of the pheromone blend is mediated by a specific set of odorant receptors, so called pheromone receptors. The tobacco hornworm Manduca sexta has become a model organism for studying insect olfaction in an ecological context. This study focuses on the molecular basis of pheromone detection. Transcripts encoding for two putative pheromone receptors have been reported previously. We analyzed expression patterns of these using full-length transcripts as basis. Male-exclusive expression of MsexOR-1 and MsexOR-4 was verified using qPCR. Both of them were exclusively expressed in males, with MsexOR-4 being more highly expressed. Topographical expression of MsexOR-1, MsexOR-4 and the olfactory coreceptor Orco was determined via double in situ hybridization. Finally we employed the *Drosophila melanogaster* empty neuron system for extensive functional characterization of the putative pheromone receptors, proving their identity. This project was funded by the Max Planck Society.

Poster session II Poster #210

Diffusion-based molecular dynamics of odorant binding in olfactory receptors

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With increasing availability of high performance computing systems, all-atom molecular dynamics (MD) simulations of olfactory receptor (OR) models in realistic chemical and thermodynamic environments are almost indispensable so that insight may be gained into the molecular basis of odor-ligand OR activation.

We performed a series of MD simulations within a solvated lipid bilayer system of the human OR, OR 17-209 (OR1G1). To further approach physiological conditions of the chemical environment surrounding an olfactory receptor, experimentally-determined (Matarazzo, et al. 2005) activating concentrations of isoamyl acetate molecules were randomly introduced into the extracellular solvent space instead of docking a single molecule into the solvent accessible binding pocket of an otherwise static receptor structure.

This novel technique allows the receptor to sample its own conformational space while the ligand molecules are free to interact with it, allowing the identification of the entry pathway into the binding pocket. In addition, systems of varying odorant concentrations and different odorant combinations can be simulated and compared to experimental results. Finally, measuring structural changes in the OR as it undergoes the transition from inactive to active conformations helps to further elucidate the mechanism for receptor activation and odorant specificity.

References:

Matarazzo, V., Clot-Faybess, O., Marcet, B., Guiraudie-Capraz, G., Atanasova, B., Devauchelle, G., Cerutti, M., Etievant, P., and Ronin, C. Functional Characterization of Two Human Olfactory Receptors Expressed in the Baculovirus Sf9 Insect Cell System. Chem. Senses 30, 691-701 (2005)

Symposium 17 "Toward a genetic basis for human olfaction" Tuesday 26 June

Personalized olfactory perception

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The vastly growing information on DNA diversity along completely sequenced human genomes makes it possible to reassess the diversity status of distinct olfactory receptor (OR) proteins in different human individuals. Our analysis includes 405 OR intact genetic loci, i.e. such at which one or more sequenced individual had an intact open reading frame. Based on information regarding haplotypes (combinations of genetic variations along a single chromosome), obtained from the public 1000 genome deep sequencing project, we defined a total of ~4000 different full-length polypeptide variants, providing a full view of the effective OR protein repertoire of the human species. We find that each individual typically harbors 500-600 allelic variants with different coding sequence, a considerably higher number than the number of intact loci. Importantly, as the sensory neurons of the nose show allele-specific expression, the brain receives readout of all such allele types, with implication to inter-individual smell perception diversity. Further, within the 405 OR loci, we identified 239 (~60%) segregating pseudogenes (SPGs), ORs that show both intact and pseudo forms in the population. Twenty-six SPGs were as pseudogenes, hence may be thought of as "resurrected". Using a custom SNP microarray we validated 187 SPGs in a cohort of 350 individuals, and obtained initial data on the association to threshold phenotypes. Our results suggest that every individual human being has his/her own personalized OR repertoire, hence perceives the odorant universe in an appreciably different fashion. This conclusion also applies to the General Olfactory Sensitivity (GOS) phenotype, with its extreme cases of congenital general anomia and general hyperemia, likely governed byolfactory accessory genes. We have created a database of hundreds of such genes and are studying the phenotypic outcome of their variations by next generation DNA sequencing and genetic association studies.

Symposium 12 "No taste, no smell: When the chemical senses are lost " Sunday 24 June

Dysgeusia, ageusia and burning mouth syndrome

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Among human chemosensory disorders, taste disorders occur relatively rarely. Nevertheless, investigation, diagnosis and etiologies as well as treatment options for clinical taste disorders remain very limited. Within the last few years new insight into taste function has been gained and clinical knowledge as been enhanced. Easy, rapid and inexpensive testing tools have been developed which allow for a routine clinical workup in patients with taste disorders. The presentation discusses recent findings on human taste dysfunctions.

Poster session I Poster #211

Nuclear rganization of olfactory receptor genes

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The mammalian olfactory system depends on a large family of olfactory receptor (OR) genes that are expressed in a mutually exclusive manner in sensory neurons: each neuron of the nose transcribes one allele of one OR gene so that the neuron becomes specialized for the odorant-binding capabilities of the single receptor protein expressed. In mice, this system of mutually exclusive OR regulation encompasses >3000 OR alleles in the diploid genome that are distributed across >50 chromosomal locations. Recent work has pointed to a model whereby all OR genetic loci are first silenced, and then one of these loci is stochastically de-repressed. We have used DNA FISH, RNA FISH, and

immunohistochemistry to investigate nuclear organization of expressed and non-expressed OR loci. We find that most OR loci are sequestered within or adjacent to well-defined repressive nuclear compartments (chromocenters), but that the single expressing allele in each cell is always liberated from these compartments. We will report on differences in nuclear organization for various OR loci with different genomic attributes and levels of representation/expression in sensory neuronal cell populations, as well as non-neuronal cell populations. We have identified a candidate chromatin modifying protein whose expression profile and biochemical activity is consistent with an involvement in the regulation of single OR genes. We are currently using RNAi to further investigate a role for this candidate OR regulator; we anticipate being able to report preliminary results of this experiment in time for the ISOT conference this June. Finally, we are fusing OR-expressing cells in order to challenge the system (at least initially immediately following fusion) with two expressing OR loci within a single synkaryon (tetraploid) cell in order to investigate the iterative properties of OR co-regulation; this is also preliminary and ongoing work on which we also hope to report at the conference.

Poster session I Poster #41

Contextual exploitation of chemosensory information in insects: Informational and ecological constraints affect the flexibility and evolvability of decision making and host use

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Insects are often characterized by a high degree of specificity in their host use; most herbivorous insects are for example constrained to a limited range of related host plants. Based on an analysis of the ecological costs associated with different types of decisions, and the value of the information available to insects in a given situation, we may draw general conclusions about the degree of flexibility and evolvability in insect foraging and host use that are valid among a broad range of insect taxa. Attraction studies show that wild-type *D. melanogaster* display relatively little attraction to single fruit and fermentation volatiles, in comparison with a more complex natural blend. Nevertheless, some non-selective strains responded to a range of fermentation related compounds, suggesting that acceptance of suboptimal stimuli was a common denominator rather than preferences for any particular cue. Generalized to a broader host selection context, this may illustrate the potential costs associated with lower selectivity in olfactory preferences. All strains displayed much higher selectivity in their oviposition preferences, however, responding only to the complex yeast odour. Comparisons between attraction preferences and oviposition preferences to a range of complex fruit-yeast stimuli revealed that olfactory preferences in attraction and oviposition are likely correlated, although with very different acceptance thresholds. This may be an effect of stabilizing selection rather than direct coupling of the two behaviours, however, as one *Drosophila* strain displayed de-coupled attraction and oviposition preferences. Subtractive selection experiments, where flies were selected for attraction or oviposition preferences to individual compounds among a general odour background, suggest that the innate preferences for individual odours respond very slowly to selection. Among the two selection regimes, oviposition preferences appear to be the most resistant to change.

Poster session I Poster #119

Characterization of vomeronasal receptor families in the deer mouse *Peromyscus maniculatus*

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Chemical communication by means of pheromones is central to the mating systems of a wide range of organisms. Since mate choice and subsequently, reproductive isolation, are often based on pheromone differences, understanding how pheromone systems diverge is necessary for a complete understanding of the evolutionary process. Because

chemoreceptors are the first elements in a cascade of events ultimately modulating behaviors, changes that affect their biochemical properties or expression pattern have the potential to modulate specific behavioral responses. In particular, the activation of sensory neurons in the vomeronasal organ (VNO) is directly associated with behavioral changes, such as aggression, avoidance or mating. The aim of this study is take advantage of recent advances made in our understanding of the molecular bases pheromone and semiochemical detection in the laboratory mouse Mus musculus and apply that knowledge to understand the evolution of pheromone systems in wild populations. To achieve this goal, we are studying deer mice (genus Peromyscus), which diverged from Mus approximately 25 MYA. Here we report on the characterization of vomeronasal receptors (VRs) from one species, P. maniculatus.

We find striking differences in VR repertoire between these two rodent species. First we queried protein sequences of all predicted M. musculus V1Rs and V2Rs against the P. maniculatus genome. In total, we identified 150 and 90 putative V1R and V2R genes, respectively, in the Peromyscus genome, fewer than in M. musculus (239 and 120, respectively). While clades previously identified in the Mus have representatives in Peromyscus, our phylogenetic reconstructions indicate that most gene duplications took place after the split between the two lineages, with few orthologs between species. In V1Rs, several clades show sign of expansion (i.e. V1Re, V1Rl) or contraction (i.e. V1Ra, V1Rc, V1Rd). In V2Rs, many expansions are lineage-specific with several clades identified in Mus having few or no representative in Peromyscus and vice versa. The differences in the chemosensory receptor repertoires likely reflect difference in habitat as well as social and mating behavior of deer mice. Ongoing RNA-Seq experiments will determine which of these receptors are expressed in male and female adults and which will be targets for functional characterization.

Poster session II Poster #330

Taste sensitivity in weight excess and management

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The role of taste thresholds in the physiopathology and the management of overweight and obesity has been scantly investigated and the few data available are rather contradictory. We investigated the relationship between taste sensitivity and nutritional status. The possible implications on the outcome of a weight loss program have been also considered. Sensitivity for bitter, sweet, salty and sour tastes was assessed by the three-Alternative-Forced-Choice (3-AFC) methodin 41 overweight (OW) and 52 obese (OB) patients and 56 normal-weight matched controls (age range 18-64 years). OW and OB were assessed also for body composition (by impedence), resting energy expenditure (REE by indirect calorimetry) and presence of metabolic syndrome (MetS) and were prescribed a weight loss diet. Adherence and compliance to the program were defined as drop-out since the next visit and weight loss of at least 5% in 3 months, respectively.

Sex and age-adjusted linear regression analysis revealed a direct independent association between BMI and the threshold for sour taste. This association was also confirmed after restricting the analysis to OW/OB group. Similarly, MetS was associated with lower sensitivity for salt even after adjusting for BMI. Finally, early drop-out (n=29) and successful weight-loss (n=37) appeared unrelated to sensory capabilities. In conclusion, although taste sensitivity appears in some measure related to weight excess and metabolic derangements we cannot argue for a cause-effect relationship. Moreover, the assessment of taste thresholds does not seem useful in predicting the outcome of a diet-induced weight loss program.

Poster session II Poster #42

Biogenic amines drive the adaptation of *Drosophila* sechellia to its toxic host.

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The evolutionary force that drove the adaptation of *Drosophila sechellia* to its toxic host fruit *Morinda citrifolia* has so far been elusive. *D. sechellia* is highly specialized in detecting and coding key components present in *Morinda* fruit, which

in turn influences the physiology and behaviour of the fly: lack of avoidance of the host toxin and an enhanced sensitivity and preference to host odours. Concomitantly, these volatiles and other yet-unknown compounds present in *Morinda* have a stimulatory effect on the poor egg-laying capability observed in female *D. sechellia* fed upon standard synthetic diet. We found that the reduced reproductive performance of *D. sechellia* under laboratory conditions correlates with decreased levels of biogenic amines in the ovary of the fly. The low egg production stems from oocyte development being halted at vitellogenic stages. Vitellogenesis normally proceeds with the biosynthesis of the oocyte vitelline membrane and chorion, for which biogenic amines are required. A *Morinda* diet re-establishes the levels of dopamine in *D. sechellia* ovaries and stimulates full oocyte development. In addition, when the precursor of dopamine, L-DOPA, is provided as dietary supplement, it also rescues oocyte development and increases egg-laying. In parallel, *D. sechellia* shows altered expression of several genes involved in dopamine metabolism as compared to its generalist siblings *D. melanogaster* and *D. simulans*, suggesting an obligate diet acquisition. And indeed, both L-DOPA and dopamine are present in *Morinda* fruit, as detected by mass spectrometry and HPLC. These results form the basis for a hypothetical scenario where the requirement of biogenic amines for successful reproduction drove the host-shift of *D. sechellia* to such a degree that it is now exclusively confined to its toxic host.

Poster session I Poster #329

Impact of expectation on taste perception

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One of the key drivers for Food Industries is to bring healthier food to the market; the key targets being salt, sugar and fat reduction. However, to achieve these targets, the composition of a product has to change, which might affect the taste of the product. Step by step reductions can lead to a certain level of reduction without major perceptual impacts on the product; but there are still progresses to be made to achieve the targets (e.g. 5-6g of salt intake per day).

It is known that previous experience with a product and the memory from this experience shapes expectation and will impact on subsequent eating experiences of similar products (De Wijk et al., 2004). When subjects assume that multisensory sensations comes from a single source, they tend to perceive them as one unit (Welch & Warren, 1980) despite spatial or temporal discrepancies existing between sensations.

Using such top-down evidences, we conducted a series of experiments with the aim to investigate if the expectation, or implicit assumption, that taste is consistent across mouthfuls can reduce the perception of variation in taste. In a first study, we demonstrated, both with a trained panel and a consumer panel, that a layered distribution of salt in a model sandwich (high level in 1st, low in 2nd, high in 3rd bite) was perceived as overall more salty than a homogeneous distribution of the same salt content. Our second study showed that the effect was not due to adaptation as we could demonstrate the reverse effect: a sequence of three bites containing a low level of a bitter compound in bite 1, followed by a high level in bite 2 and a low level again in bite 3 was perceived as overall less bitter than a sequence of three bites containing the same total amount of this compound distributed homogeneously over the three bites. This set of experiments and their findings could influence applied research aiming to make foods healthier while optimizing taste.

Poster session I Poster #43

Biochemical crowdsourcing through oral fluid-exchange in ant colonies

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Communication is essential in high-functioning social groups, from social insects to humans. How do ants bring about

colony-wide change without language or top-down control? While ants are traditionally thought to communicate mostly through pheromones, we are testing whether trophallaxis – a method of mouth-to-mouth liquid transfer – may also be an important pathway for communication enabling a form of chemical crowdsourcing. Given the power of trophallaxis to rapidly distribute liquids throughout the colony, trophallaxis would provide an excellent means of information transfer, especially for compounds unstable outside the body. Socially exchanged fluids, e.g. seminal fluids, often carry ancillary information that alters behavior. To date, the contents of trophallaxis fluids have not been thoroughly analyzed and thus the precise function(s) of this striking behavior remain unknown.

Using nano-liquid chromatography tandem mass spectrometry, we have biochemically analysed the protein components passed between individual ants during trophallaxis. In addition to the anticipated insect gut proteins, we have also found proteins potentially involved in the hormonal regulation of social insect behavioral maturation – the age-related transition from nurse to forager. A number of proteins passed between nestmates appear to vary depending on the social caste of the donor and have high sequence similarity to well-known insect hormonal regulators, suggesting that by passing regulatory molecules to one another, nestmates might influence one another's behavioral maturation. These biochemical studies are being paired with a highly quantitative, barcode-based ant tracking system to measure the behavioral causes and effects of trophallaxis and the long-term behavioral effects of candidate proteins.

Poster session II Poster #44

Modulation of pheromone communication in *Drosophila melanogaster*

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Drosophila flies gather on overripe fruits, where they feed, mate and lay their eggs. In order to find a mate, a male should locate fruits on which other flies, and especially females, are present. We investigated the role of food-related odours and pheromones in mate finding in *D. melanogaster*. Our results show that upwind flight attraction is mainly mediated by food-related odours and modulated by starvation. However, when approaching the source, males are attracted by pheromones previously produced by copulating flies. A male-produced pheromone, *cis*-vaccenyl acetate (cVA), which is released in high amounts during mating, is responsible for this attraction. Attraction to cVA is modulated by physiological factors, such as starvation. Virgin and sexually receptive females are attracted to cVA, too. By being attracted to cVA, males can meet receptive females that are attracted to the same place. These results bring new insights into the mechanisms how fly males can find a mate and how cVA actually regulates mating in *D. melanogaster*.

Poster session II Poster #302

Training of olfactory function in old age

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As a rule, olfactory function decreases in old age. We analyzed whether the ability to smell could be improved by repeated, short-term exposure to a specific set of odorants. For this study on the effects of “smell training” we recruited

104 subjects with an average age of 81 years. Following a structured, short medical history and exclusion of dementia based on results from the “mini mental state examination”, odor thresholds for phenyl ethyl alcohol and scores in an odor identification task were determined by “Sniffin' Sticks” before and after the training. 43 volunteers participated in the training for 12-16 weeks using four different odors (phenyl ethyl alcohol [“rose”], citronellal [“lemon”], eugenol [“cloves”], and cineol [“eucalyptus”]), that had been selected according to the “smell prism” described by Henning in 1916; 49 subjects did not perform such a training. Questionnaires were used to analyze the importance of smelling and the current mood state. Results indicated that training had tendential effects on odor threshold ($p=0.096$) while it did not translate into a major change of odor identification scores. Specifically, on an individual level, in subjects performing the

training odor thresholds increased by 2 or more dilution steps in 7% and decreased in 5%; for subjects not performing the training these numbers were 6% and 14%, respectively. In conclusion, the present data indicate that "olfactory training" may help to improve aspects of olfactory function.

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Poster session II Poster #328

Computational spotlight on potential new agonists of hTAS2R14

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Human bitter taste receptor hTAS2R14 is a particularly broadly tuned receptor, with over 50 agonists known to date. We have analyzed the physicochemical properties of these molecules and compared them to properties of true negatives – i.e., molecules known not to activate hTAS2R14. This analysis provided hTAS2R14-characteristic ranges of physicochemical properties.

To identify additional potential agonists of this receptor, we compiled a pool of candidate molecules, combining established bitter compounds from the BitterDB database, with potentially bitter molecules, such as datasets of approved drugs, traditional Chinese medicines and natural compounds. This dataset of candidate molecules was filtered using the hTAS2R14-like properties ranges, resulting in a subspace of candidate molecules that may potentially activate hTAS2R14.

Next, ligand-based and structure-based pharmacophore models of hTAS2R14 activators were constructed and used to prioritize the candidates subspace. Among the predicted agonists we found Guaifenesin, a non-prescription drug used to reduce chest congestion caused by the common cold, infections, or allergies. Preliminary results using functional assays of hTAS2R14-transfected HEK-293 cells confirm that several of the predicted substances were indeed positive and are potential novel hTAS2R14 agonists.

The virtual screening of bitter taste receptor agonists presented here illustrates an efficient alternative to high throughput screening approaches and provides a route to assign bitter molecules to their receptor targets.

Poster session I Poster #331

Similarities and differences in bitterness recognition by taste receptors

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Bitter-taste perception in humans is mediated by 25 GPCRs of the hTAS2R gene family. How can merely 25 receptors detect thousands of structurally diverse bitter compounds, and why some of the receptors are broadly-tuned, while others are capable of binding only a small number of ligands? The structural basis for the bitter taste receptors unique ability to specifically allocate numerous chemically diverse agonists is the focus of the current study.

A crucial step in understanding specificity and promiscuity in molecular recognition is to identify residues important for ligand binding. To elucidate the sites of interaction between the bitter taste receptors and their different agonists, we generated all-atom 3D models of the receptors, based on solved X-ray structures of family A GPCRs. Binding site prediction was preformed by an energy-based method. Computational docking of structurally distinct agonists to the receptors was used to evaluate feasibility of the predicted specific interactions and followed by *in vitro* assays to confirm the proposed binding mode.

This approach was applied to two broadly-tuned human bitter taste receptors – hTAS2R10 and hTAS2R14. Functional assays on wild-type vs. mutant constructs confirmed the *in silico* predicted interactions and corroborated the main predicted binding site, situated inside the trans-membrane bundle and analogous to sites in related receptors. Interestingly, both receptor and ligand-specific interactions were revealed, illustrating the complexity and versatility of the GPCR transmembrane pockets.

The iterative scheme for elucidation of molecular recognition of the bitter compounds by their cognate receptors represents first steps towards *in silico* bitterness prediction.

Poster session II Poster #324

Identification and characterization of salt-responsive amiloride–insensitive taste receptor cells

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Salt taste plays an important role in determining dietary sodium intake, which has implications for human health. Mammals are known to have two salt-sensing mechanisms: 1) amiloride-sensitive, mediated by the epithelial sodium channel (ENaC), and 2) amiloride-insensitive. Little is known about the molecular and cellular mechanisms of the amiloride-insensitive component of salt taste. Even the cell types within the taste bud that express amiloride-insensitive salt taste receptors have yet to be definitively identified. Here we used calcium imaging and single-cell molecular profiling of dissociated mouse taste cells to identify and characterize cells expressing amiloride-insensitive salt taste receptor(s). We demonstrate that amiloride-insensitive NaCl responses (250 mM NaCl, 30 μM amiloride in Tyrode's solution) are only observed in a subset of the taste cells that respond to KCl depolarization with a transient elevation of cytoplasmic calcium. This suggests that the amiloride- insensitive salt taste receptor is likely to be expressed in Type III taste cells. This observation was confirmed by analysis of single-cell transcripts. Overall, our data demonstrate that amiloride- insensitive sodium taste receptor(s) are expressed in Type III taste cells. This observation provides a foundation for determining the molecular identity of the amiloride-insensitive sodium taste receptor.

Poster session I Poster #369

Identification of enterodiol as a potent bitter masker by combined gustophor modeling and docking studies

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Natural products showing a strong bitter masking effect are of great value for development of functional food as well as for the elucidation of the bitter reception mechanisms in general. During the last years, our group was able to identify several bitter masking flavanons and related molecules by sensory screening using caffeine (*1-3*) as a prototypical bitter elicitor activating several human bitter receptors in vivo (*4*). Some of the found bitter inhibitors, e.g. homoeriodictyol, showed a surprising broad masking effect against various other bitter compounds. Therefore, it also seems to interact with one of the more broadly tuned receptors.

Sensory screening is of high value to get a holistic taste impression but is self-limiting due to the low-throughput approach and the limited amounts available of most natural products. To accelerate the process of identification of potential natural bitter masking compounds, we have (i) developed a gustophor model based on structure-activity relationships of previously described structural classes (*1-3*); (ii) performed a binding study of the best masker homoeriodictyol on one of the broadly tuned human bitter receptors including a validation with the gustophor model; (iii) virtually screened a test set of natural products without any reported sensory data; and finally (iv) evaluated the potential candidates using our standard masking protocol.

Using this approach, we were able to identify the natural occurring enterodiol as a potent caffeine masker. The structural analogue enterolacton was predicted to be not active and indeed the molecule not only had no masking but a strong bitter enhancing effect in the sensory tests.

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Poster session II Poster #434

Disruption of TGF—β signaling promotes squamous cell carcinomas in lingual epithelia

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The transgenic Keratin14-rtTA-PTR mice specifically express Keratin14 in the tongue epithelia and also co-express EGFP and the dominant negative ΔTgfbr2 genes upon treatment with Dox. As TGF-β signaling negatively regulates the stem cell cycle and proliferation, its disruption by Doxycycline (Dox) induction in these transgenic mice shortens the cell cycle and allows observation of the final fate of those mutated cell lineages within a short period of time. Here, we used inducible transgenic mice to track the Keratin14+ cells through the cell migration stream by immunohistochemical and immunofluorescent imaging. We showed that these cells have different development patterns from the tip to posterior of the tongue, achieved presumably by integrating positional information from the microenvironment. The expression of the Keratin14 gene was variable, depending on the location of the tongue and papillae. Disruption of TGF-β signaling in Keratin14+ progenitor cells resulted in proliferation of stem cell pools. Finally, we showed that mice lacking TGF-β signaling in K14-positive cells developed invasive squamous cell carcinomas on ventral surface of tip tongue. By contrast, Tgfbr2 mutant lingual epithelium on dorsal surface was phenotypically normal, and although filiform papillae showed the different pathological changes from tip tongue to posterior tongue. In addition, the acetylation level of histone H4 and histone H3 was rapidly elevated in lingual epithelia, Jagged2 was inactivated after disruption of TGF-β signaling. In short, this study highlights the important roles of the microenviroment and epigenetic modifications during the developmental process of tongue epithelial stem cells. Our data also provide a mechanistic framework for understanding the role of TGF-β signaling in regulating homeostasis and carcinogensis in lingual epithelia.

Poster session II Poster #370

The taste detection threshold for sucrose in dopamine D3 receptor deficient mice

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Some evidence suggests intake of sweet foods is affected by dopamine related signals. The preference for sweet foods may involve dopamine release changes in response to the taste of sweets. Our recent work showed that dopamine D3 receptor deficient mice (D3-/- mice) consumed significantly less sucrose solution at the concentration of 0.1 and 0.005M without any preference for less concentrated sucrose solutions changed. To test the possibilities that whether D3-R interferes with the ability to detect dilute sucrose solutions, we conditioned a taste aversion to 0.2 M sucrose in D3-/- mice by pairing it with injection of LiCl and then examined the generalization of that taste aversion to 0.1 and 0.0001 M sucrose solutions. Wildtype mice generalized the LiCl-induced aversion conditioned to 0.2 M sucrose to a concentration range from 0.1M to 0.005 M sucrose. D3-/- mice generalized the LiCl-induced taste aversion to a concentration range from 0.1 M to 0.05 M sucrose. To further preliminarily clarify the possible molecular mechanisms underlying this change, we measured gene expression for the sweet taste related signals in taste buds of D3-/- mice and found that there are increased gene expression of T1R1 and BDNF in D3-/- mice without any observed alterations in the gene expression of T1R3, T1R2, gustducin and TRPM5. These results show that dopamine D3 receptor affects the ability to discriminate dilute sucrose from water and alter the gene expression of T1R1 and BDNF. These processes may be involved in the mechanisms underlying the modulation of peripheral sweet taste sensitivity in D3-/- mice.

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Poster session I Poster #11

Protocerebral projection patterns of antennal-lobe out-put neurons originating from the MGC and the ordinary glomeruli in the *Heliothis virescens* male.

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Male moths have developed a distinct neuronal pathway for processing information about female-produced compounds, termed the pheromone pathway. This male-specific system includes a group of receptor neurons converging onto an easily identifiable structure in the primary olfactory center of the brain, the so-called macroglomerular complex (MGC). An additional assembly of relatively numerous glomeruli, the so-called ordinary units, receives input from receptor neurons detecting plant odours. The arrangement of ordinary glomeruli seems to be equivalent to the system described in females. From the primary olfactory centre, the information is mediated mainly via three parallel tracts the medial, the mediolateral, and the lateral antenno-protocerebral tract projecting to partly overlapping areas in the protocerebrum. The two major target regions are the calyces of the mushroom bodies and the lateral protocerebrum. As compared to the relatively well explored chemotopic organization characterizing the first synaptic level of the olfactory pathway, less is known about the encoding principles residing within the higher olfactory centres. By using a technique where two different fluorescent dyes were applied to the same brain preparation, one in the MGC region of the antennal lobe and the other in the area of ordinary glomeruli, we have visualized the protocerebral regions targeted by the pheromone and the plant-odor system. Among the obvious differences found between the two pathways are the distinct projection patterns appearing in the calyces the mushroom bodies.

Poster session II Poster #440

Human-like odor perception of spoiled seafood using an olfactory receptor-derived peptide-based bio-mimetic artificial nose

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Human percepts specific smell generated from spoiled seafood through its own olfactory system. This specific smell perception is triggered by the binding event between odorants from spoiled seafood and olfactory receptors expressed on the surface of olfactory neurons. In order to perceive the odor of spoiled seafood in vitro by mimicking the biological olfaction, we have developed an artificial nose using an olfactory receptor-derived peptide (ORP) fused with carbon nanotube-field effect transistors (CNT-FETs). ORPs, immobilized onto the CNT-FETs, selectively recognized trimethylamine (TMA) which is a major component in the odor of spoiled seafood. And then, the recognition was transduced and amplified to electrical signals by CNT-FETs. Since ORP-based artificial nose has the ability to sensitively and selectively discriminate 10 fM amount of TMA, it allowed us to specifically detect TMA in four types of spoiled seafood (oyster, shrimp, lobster, and king crab) without any pretreatment processes. We verified that the artificial nose was able to not only selectively recognize the odor from spoiled seafood among the odors from other types of spoiled foods, but also determine the degree of decomposition of seafood. Considering these facts, our artificial nose well mimicked the odor perception system of spoiled seafood, and can be applied for the rapid and easy on-site determination of seafood quality.

Poster session II Poster #212

Sox2 suppresses neuronal fate while permitting progenitor proliferation in the olfactory epithelium.

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The mammalian olfactory epithelium (OE) is a powerful model for studying neural regeneration. Two broadly defined basal populations: globose basal cells (GBCs) and horizontal basal cells (HBCs), participate in regeneration after injury. GBCs have been shown to be multipotent progenitors through transplantation assays where GBCs give rise to all cell types in the OE. HBCs appear to play the role of a reserve population, which require activation through injury prior to acquiring multipotency.

We have previously shown that Pax6 and Sox2 are expressed in both GBCs and HBCs. Pax6 is known to play roles in progenitor cell multipotency while Sox2 has been extensively shown to maintain an undifferentiated stem cell-like state. Our previous work has implicated a role for Pax6 and Sox2 in suppressing neuronal differentiation. Retroviral mediated over-expression of Pax6 and Sox2 separately and together have shown the complex interplay between the two genes. Pax6 affects cells as predicted: by markedly reducing the likelihood that a stem/progenitor cell gives rise to neurons and limiting their number if the stem/progenitors escape that block. In contrast, Sox2 seems to play a more complex role; fewer progenitors make neurons, but those that do produce a larger number of them. To assay whether any of the Sox2 phenotype is attributable to the HBC population specifically, we accomplished conditional knockout of Sox2 in HBC's by generating a Sox2 $^{fl/fl}$ ::K5-CreER mouse. Genetic elimination of Sox2 in the context of recovery after MeBr confirms that the loss of Sox2 in HBCs results in a population shift away from neurons and towards HBCs, as well as smaller clones in general. When all the data are combined, we are able to show that Sox2 permits proliferation of HBCs while suppressing the downstream neuronal fate.

Poster session I Poster #435

Beyond the shape theory of olfaction: Inelastic electron tunneling in olfactory receptors

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The smell of an odorant molecule is determined by its ability to activate an array of olfactory receptors, with each unique scent activating a unique set of receptors. The mechanism behind this activation is not well understood. An odorant's size and shape matter, but a simple lock-and-key explanation may not be sufficient. An alternative proposal is that activation is due to phonon-assisted tunneling in the receptor. We combine analytical modeling and numerical quantum chemistry approach to compute the signal transition rate and find it a plausible mechanism for olfactory receptor. The approach developed here provides quantitative differentiation between odorants and their isotopes, serving as the best test for the vibration hypothesis. In addition, our model reveals that quantum theory may play an essential role in olfactory detection.

Delwart Contributed Symposium - Higher olfactory processing Tuesday 26 June

Bulbar acetylcholine modulates cortical associative learning

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The olfactory bulb and piriform cortex are the recipients of dense cholinergic projections from the basal forebrain. In the present work we implement a computational model of olfactory bulb (OB) and piriform cortex (PC) and their common cholinergic inputs to investigate how bulbar muscarinic signaling regulates the rate at which the olfactory system learns odor representations. We show that blocking muscarinic receptors in granule cells drastically decreases synchronization of mitral cell output without affecting overall firing rates. In the PC, pyramidal cell (Pyr) firing depends on the co-activation of many glomeruli, as shown experimentally (Davison and Ehlers, 2011). In our model, less synchronized input patterns reduce the overall activity of Pyr cells and, as consequence, decrease the learning rate of odor patterns in the cortical associative memory network. We next performed a set of pharmacology experiments in order to test the model's predictions. Adult, male Long Evans rats (n=4) were trained in a two-alternative (go-left, go-right) forced-choice olfactory discrimination task. Each week, rats completed five consecutive daily sessions using a novel odor set. Criterion performance was defined a two consecutive sessions with >70% performance. We evaluated the influence of bulbar muscarinic signaling on the task by comparing performance in rats receiving infusions of 0.9% saline versus 38 mM scopolamine bilaterally into the olfactory bulbs. Control rats easily acquired novel discrimination problems, requiring an average of 2.5 sessions to criterion performance. Consistent with model predictions, blocking muscarinic signaling in the OB significantly decreased the learning rate, with scopolamine-infused rats requiring an average of 4 sessions to reach criterion performance.

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Poster session I Poster #45

Olfactory adaptations in the "seasonal" specialist *Drosophila* erecta towards its host

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Many insects primarily rely on the sense of smell to orient within their environment. The olfactory systems of insect species should, therefore, reflect adaptations to specific hosts. The vinegar fly *Drosophila erecta* is remarkable for its specialization for feeding and breeding on ripe fruits of screw pine trees (*Pandanus* ssp.) in gallery forests of west central Africa. The species belongs to the *Drosophila melanogaster* species subgroup, which originated in tropical Africa. Besides a close relationship, the subgroup shows a considerable interspecific variation - ranging from extreme specialists to generalists, and from endemic to cosmopolitan species. Our study addresses the question whether the specialization towards its host fruit has caused changes within the olfactory system of *D. erecta*. To study the effects of this host specialization we compared several co-occurring *melanogaster*-group sibling species with different host specificity and ecology. By performing linked gas chromatography – mass spectrometry – electrophysiology of *Pandanus* fruit headspace volatiles we identified biologically active components guiding the flies to their oviposition sites. Our results let us assume that non-specialist siblings differ in their olfactory response from the specialists. Furthermore, we found a new fruit-derived component, which might play a major role in host identification of *D. erecta*. Immunohistological analysis and optical imaging studies of the olfactory system confirm our findings by revealing a shift within the first odour-processing centre.

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*contributed equally

Poster session I Poster #189

Faster, deeper, better: The impact of sniffing modulation on bulbar olfactory processing

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A key feature of mammalian olfactory perception is that sensory input is intimately related to respiration. Different authors have considered respiratory dynamics not only as a simple vector for odor molecules but also as an integral part of olfactory perception. Thus, rats adapt their sniffing strategy, both in frequency and flow rate, when performing odor-related tasks. The question of how frequency and flow rate jointly impact the spatio-temporal representation of odor in the olfactory bulb (OB) has not yet been answered. In this study, we addressed this question using a simulated nasal airflow protocol on anesthetized rats combined with voltage-sensitive dye imaging (VSDi) of odor-evoked OB glomerular maps. Contrary to previous studies based on presynaptic activity measurements, VSDi signals take into account possible reshaping of incoming activity by local interneurons.

Experiments were performed on urethane anesthetized rats. The OB was stained with voltage-sensitive dye RH 1838 and image series of the dorsal OB were acquired with a CCD camera at 160 Hz. We used a double cannulation protocol in order to make nasal airflow sampling independent from animal respiration. Thanks to this technique, we were able to mimic actual sniffing with an independent control of flow rate and frequency within the physiological range. Glomerular responses displayed a tonic component during odor stimulation with a superimposed phasic component phase-locked to the sampling pattern. We showed that high sniffing frequency (10 Hz) retained the ability to shape OB activity and that the tonic and phasic components of the VSDi responses were dependent on flow rate and inspiration volume, respectively. Both sniffing parameters jointly affected OB responses to odor such that the reduced activity level induced by a frequency increase was compensated by an increased flow rate.

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Symposium 23 "Evolution of chemosensory systems " Wednesday 27 June

The genetic complexity of olfaction in mice: an evolving story

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The sequencing and annotation of a mouse genome was undoubtedly a landmark in mouse genetics, and revealed a remarkable repertoire of genes underpinning olfaction in mammals. But it soon became clear that a single genomic snapshot cannot resolve the true genetic complexity of the large, homologous gene families encoding chemosensory receptors. We are now using next-generation sequencing to fully interrogate the olfactory systems of mice in depth and breadth, at both genomic and transcriptomic levels.

We have sequenced the genomes of 17 strains of mice and compared variation in chemosensory receptor repertoires. We identify unusually high amounts of variation, non-randomly distributed within and between single and clusters of receptor genes. We also find receptor content differs considerably between the genomes of closely related *Mus* subspecies and species, suggesting some chemosensory receptors may play a key role in mediating specific adaptations while others are highly conserved within the genus.

In parallel, we have characterized the olfactory transcriptomes of lab mice by RNA sequencing, identifying new full length transcripts, alternative splice forms, sexual dimorphisms and the accurate expression levels of over 36,000 unique genetic elements. We have further investigated a few highly expressed genes not previously implicated in olfaction using gene-targeting, and also identified some entirely novel abundant transcripts not yet annotated in the genome. We compared the expression levels of hundreds of chemosensory receptors and found they are unequally expressed across a large dynamic range. However, abundances are highly consistent between individual mice of the same strain, suggesting there is a non-stochastic selection bias in sensory neurons for some receptors.

We have made these sequence data and mouse lines immediately publicly available to encourage community

investigation into the olfactory consequences of this genomic and transcriptomic diversity.

Poster session II Poster #282

Human neonates' responses to the odour of high dilutions of androstenone

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Studies dealing with mammalian newborns, including human neonates, suggest a transnatal chemosensory continuity between amniotic and lacteal fluids. This continuity is assumed to be one of the processes promoting the success of initial milk intake, critical for neonatal survival. Several odorants have been detected in both human amniotic fluid and colostrum/milk, thereby possibly underlying the transnatal odour continuity. Amongst others, 5-α-androst-16-en-3-one (AND), also contributing to human sweat odour, has been identified in both biological fluids. As it has been demonstrated that the human fetus is able to learn and retain single odorants of his environment, we supposed AND could function as a vector of relative similarity between prenatal and postnatal environments. Previous tests having shown negative facial expressions to higher AND concentrations, the experiment presented here aimed to assess the newborns' responses to picogram-wise AND concentrations.

We observed the reactions of 3-days-old newborns (n=32, 16 females) in episodes of active sleep during (10 s) and after (10 s) orthonasal presentation of 3 AND solutions (0.5, 5, and 50 pg/L) and an odourless control (water). The respiratory rate and amplitude, as well as the duration of facial movements, of the newborns were quantified to assess olfactory perception and hedonic reactivity.

The results suggest that the AND concentrations used here appeared "peri-liminal" for the newborns. With regard to the respiratory rate and facial expressions, the neonates did not differentially respond to the AND solutions and the odourless control. Yet, the highest AND concentration used affected the respiratory response in the sense that the respiratory amplitude increase was more pronounced in boys than in girls. This would suggest that neonate boys are more reactive or sensitive to AND than neonate girls. This result is in line with that of previous tests using higher AND concentrations.

Poster session II Poster #414

Insect-based odor localization and classification on an indoor autonomous robot

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Insects are capable of performing robust odor classification and localization in turbulent environments. In robotics, however, odor localization and classification is still a challenge. In our work we embraced a biomimetic approach and applied the principles of insect behavior and its underlying neuronal structures to an autonomous robot that has to identify and locate a desired odor source in a wind tunnel.

We have developed two separate models performing odor localization and classification tasks using the large scale neural network simulator IQR [Bernardet & Verschure (2010)]. The classification model analyzes the signal from the sensors and uses the, so called, Temporal Population Code (TPC) to process real-world real-time chemosensor input combined with a classifier that approximates the role of the mushroom body kenyon cells to identify the odor captured at each moment. The localization model, based on the cast and surge odor search strategy of the moth, controls the robot's movements and determines its behavior in relation to the odor detected by the classification model.

We have performed two different experiments: 1) static, in which the robot was not moving and only the classification

model was executed; and 2) dynamic, when both models were running and behavior was added by the localization model. Odor maps have been reconstructed from the static experiments, while trajectories, distance covered, and time elapsed has been analyzed from the dynamic ones. Moreover, we compared the performance of the classifier in sensitivity and specificity terms between static and dynamic experiments.

Our results show that the combined use of the localization model and the classifier leads to robust localization of the sources of two simultaneous odor compounds in the environment. These results demonstrate the role that dense spatio-temporal codes can play in the real-time processing of chemical signals by active sensing systems such as insects and robotics.

Poster session II Poster #46

Molecular characterization of the olfactory receptors of *Rhodnius prolixus*, a vector of Chagas disease

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Insect olfactory neurons house two types of odour receptors: G protein-coupled receptors (ORs) and ion channel formed receptors (ionotropic receptors, IRs). Both types require the co-expression of specific co-receptor proteins in order to be functional: OrCo (for ORs) and IR25a or IR8a (for IRs). Our objective was to characterize ORs, IRs and olfactory co-receptors at the molecular level in *Rhodnius prolixus*, an important vector of Chagas disease. First, receptor sequences from other insects were chosen by searching genomic databases. These sequences were then compared with predicted proteins from the *R. prolixus* genome (unpublished data), allowing the identification of homologous sequences. The programs Wise2 BioEdit and MEGA ClustalW2 were used to analyze candidate sequences and compare them with those of other insects. Through applications available on www.cbs.dtu.dk/services/, characteristic structures, including channel and transmembrane domains, were identified. Bioinformatic analyses on the sequences and structures allowed confirming the identification. Through the IDT Primer Quest program, specific primers were designed to allow sequencing the three co-receptors. New primers were designed to study co-receptor expression (OrCo and IRs) in different tissues (antenna, proboscis, front, mid and hind tarsi, genitalia and fat body) by RT-PCR. Results showed antenna-specific expression of several ORs and two co-receptors, IR8a and OrCo. However, IR25a showed widespread expression, as already described for other insects. Finally, OrCo transcription levels were measured in males and females of different ages by qPCR. The expression of IR8a and OrCo confirms their relationship with olfactory processes. However, the expression of IR25A outside antennal tissues suggests that it could be involved in other functions. A better understanding of triatomine olfaction may reveal targets for developing new vector control tools.

Contributed talks III "Mixed session" Monday 25 June

Casting light on the interplay between perception and decision making in *Drosophila* chemotaxis

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Active sensing couples motor responses to sensory processing in feedback loops that can be challenging to investigate experimentally. We combined high-resolution behavioral analysis, electrophysiology, modeling, and optogenetics to dissect the sensorimotor integration underlying chemotaxis in *Drosophila melanogaster* larvae.

When exposed to a static odor gradient, larvae orient through a series of runs punctuated by turns. The timing and

directionality of turning events proceed from active sampling. Prior to turning, the local odor gradient is resolved through side-to-side head movements (casts). Larvae genetically engineered to retain function in a single olfactory sensory neuron (OSN) demonstrate the same basic orientation strategy as wild type. We reconstructed the sensory dynamics that the larva experiences at key decision points during unconstrained chemotaxis. Using electrophysiology, we recorded the responses of the single functional OSN to a replay of the stimulus time course. Two types of signals were characterized in detail: rapid fluctuations in concentration associated with sub-second head casts, and slower odor ramps detected during runs lasting several seconds. These neural responses constitute the mechanistic basis for a model underlying the spatiotemporal integration of dynamical olfactory inputs and its conversion into orientation responses.

Our sensorimotor model was tested in virtual reality experiments. Using light sequences to trigger controlled activity patterns in a single-functional OSN expressing channelrhodopsin, we can induce predictable changes in behavior in response to well-defined sensory inputs delivered during specific behavioral states. We exploited this closed-loop paradigm to establish a relationship between the neural integration of sensory evidence and the probability of implementing stereotyped motor responses. Overall, our work clarifies how a simple brain uses active sensing and decision making to direct behavior.

Contributed talks IV "Olfactory receptors, ligand interactions and transduction mechanisms" Monday 25 June

Odorant activation of insect olfactory receptors is inhibited by antagonism of the odorant receptor co-receptor subunit

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Insects detect attractive and aversive chemicals using several families of chemosensory receptors, including the OR family of insect odorant receptors, making these receptors appealing targets for the control of insects involved in disease propagation and agricultural damage. Insect ORs are odorant- gated ion channels, consisting of two essential parts: an odorant binding subunit and a co-receptor subunit (Orco). VUAA1 (CAS: 525582-84-7) was recently identified as a novel OR agonist that acts directly on Orco (Jones et al., 2011, PNAS 108: 8821-5). We screened ORs from several insect species (*Drosophila melanogaster*, *Culex quinquefasciatus*, and *Ostrinia nubilalis*), using heterologous expression in *Xenopus* oocytes and electrophysiological recording, with a panel of compounds (termed Orco Ligand Candidates, OLCs) that were structurally related to VUAA1 (4- Ethyl-1,2,4-triazol thioacetamide core with alterations to the pyridine and phenyl rings) or similar to portions of the VUAA1 structure. OLC3 (pyridine nitrogen moved to the 4 position) and OLC12 (pyridine nitrogen in the 4 position and a 4-isopropyl moiety on the phenyl ring) were identified as agonists of the Orco subunit. We also identified a series of antagonists, each able to competitively inhibit OLC12 activation of Orco. A similar pattern of agonist and antagonist sensitivity was displayed by Orco subunits from different species, suggesting a highly conserved binding site structure. Importantly, we found that Orco antagonists could inhibit odorant activation of insect ORs through a non-competitive mechanism. Susceptibility to inhibition of odorant activation by Orco antagonism was conserved across disparate insect species, identifying Orco antagonism as a promising target for the development of novel, broadly active insect repellants.

Symposium 7 "Human olfaction" Sunday 24 June

When sensory systems merge, congruency matters

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The everyday sensory experience of our surroundings consists of a complex mix of overlapping sensations originating from various sensory modalities, each conveying very different qualitative impressions of the world. Not only is each sensory modality conveying unique information, but each alters, and sometimes distorts, the others' messages in a complex manner, about which little is currently known. Despite the disparity of these sensations, we are nevertheless able to

maintain a coherent and unified perception of our surroundings. Behavioral studies have demonstrated that sensory integration occurs when the stimuli are congruent in their spatial and temporal presentation. However, an arguably more important aspect of sensory integration is learned congruency, or so-called semantic congruency. Semantic congruency, i.e. when two stimuli of different modalities are perceived as originating from the same semantic object, is predominantly based on repeated pairings of two or more stimuli until an association is formed. Data presented here will explore the specific effects of semantic congruency on crossmodal sensory integration of visual-olfactory stimuli. Combining psychophysical measures with various neuroimaging (EEG & fMRI) and neurostimulation tools (rTMS), we have found that crossmodal olfactory-visual stimuli perceived as semantically congruent are also perceived as more pleasant, being a vital part of our neural sensory integration process, and are partly regulated by primary sensory cortical areas. These experience-dependent effects are paralleled by behavioral improvements in odor performance. Our findings highlight the importance of employing congruent multisensory stimuli whenever extrapolating to ecologically-relevant situations.

Poster session I Poster #311

Neuronal activity in response to chemosensory social signals differs between women high and low in social competence

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Studies based on clinical populations such as schizophrenia or autism have shown a low level of social competence to be related to deficiencies in social perception. As human body odors pose significant social signals, the current study addressed differences between healthy individuals high and low in social competence in the functional processing of human body odors. Twelve women low in social competence (LS) and 14 women high in social competence (HS) underwent fMRI during presentation of human body odors. In general, body odors activated brain areas known to be involved in the processing of human chemosignals (amygdala, insula, thalamus, orbitofrontal cortex). HS compared to LS individuals showed more pronounced activation of the inferior frontal gyrus and the caudate nucleus. With the inferior frontal gyrus being a crucial part of the human mirror neuron system, and the caudate nucleus implemented in the reward system, these results suggest that for HS compared to LS individuals human body odors signal the opportunity to engage in valued social interaction, and activate empathy related neuronal system which might foster competent social behavior.

Poster session II Poster #332

Cyclic-AMP regulates postnatal neural and behavioral responses to NaCl in young rats

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In rats, the epithelial Na^+ channel (ENaC)-dependent benzamil (Bz)-sensitive NaCl chorda tympani (CT) taste nerve response is not present at birth but develops between approximately 10 and 45 days of age. At present, the signaling mechanisms involved in the maturation of the (Bz)-sensitive NaCl CT responses in young rats are not known. We tested the hypothesize that an increase in taste cell cAMP is an essential step in the conversion of the CT response profile observed in young rats to the CT response profile observed in adult rats. The increase in gustatory Na^+ sensitivity with age is most likely due to a progressive addition of newly synthesized functional ENaC units or the redistribution of the α-, β- and γ- subunits from the intracellular to the apical domain. Our results show that in 19 to 23 day old rats, NaCl CT responses are Bz-insensitive but are blocked by cetylpyridinium chloride (CPC), a TRPV1t blocker. Topical lingual application of 8-CPT-cAMP (2.5-20 mM) for 10 min induced a CPC-insensitive but Bz-sensitive increase in the NaCl CT response and the apical Na^+ conductance in taste cells calculated by the increased voltage-sensitivity of the NaCl CT response post-cAMP treatment. In two bottle preference tests, 19-23 day old rats did not distinguish between H_2O and 150 mM NaCl. However, following topical lingual application of 8-CPT-cAMP, rats demonstrated clear preference for NaCl over H_2O. We conclude that the postnatal age-dependent maturation of the neural and behavioral responses to NaCl

require an increase in taste cell cAMP that presumably induces the redistribution of the α-, β- and γ- subunits from the intracellular to the apical domain.

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Symposium 1 "The other noses – the vomeronasal organ, the septal organ and the Grüneberg ganglion" Saturday 23 June

What does the septal organ tell the brain?

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The septal organ, a small patch of olfactory epithelium situated in the ventral base of the nasal septum, is a distinct chemosensory organ present in many mammals. In addition to its peculiar location in the direct air path near the nasopharynx, the septal organ shows a number of intriguing features which have important implications on its function. First, olfactory sensory neurons (OSNs) in this region show highly restricted odorant receptor (OR) selection; approximately 50% of them expresses one particular OR, named SR1. Second, as revealed by patch-clamp recordings, most septal organ neurons including SR1 cells respond to many, structurally unrelated odorants, and over a wide concentration range. The broad response profile of SR1 is further confirmed in SR1 deleted OSNs and in a heterologous expression system. Third, most septal organ neurons respond to mechanical stimuli delivered by pressure ejections of odor-free Ringer's solution. Similar mechanosensitivity also exists in ~50% of OSNs in the main olfactory epithelium and is associated with certain OR types such as SR1. Genetic ablation of key signaling proteins in odor transduction, SR1 receptor, or OR-G protein coupling eliminates mechanical responses of OSNs, revealing an exceptional case in which G protein coupled ORs serve as both chemical and mechanical sensors. By concentrating broadly responsive and mechanosensitive OSNs in the direct air path, the septal organ may serve as a general odor detector and/or an airflow monitor for the brain. Supported by NIDCD/NIH.

Symposium 11 "The stimulus – odor space and chemometrics" Sunday 24 June

Olfactory perception space analysis: merging psychophysical odor quality descriptions and chemotopic response patterns of the olfactory bulb in rodents

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Odorants bind to receptors on the surface of olfactory sensory neurons in the nasal cavity, and the differential activity of these neurons is reorganized into distinct spatial patterns in the olfactory bulb. Such patterns have been described for hundreds of odorants using the [14C]2-deoxyglucose uptake technique. Although previous studies have detected relationships between these activity patterns and odorant chemistry, the considerable overlap in patterns for odorants of different chemistry has complicated our understanding of how perceptions are extracted from these activity patterns. The same, of course, holds for odor quality. Seen from an information-theoretic point of view, we expect information on the odor quality already included in the glomerular response pattern as measured by these uptake techniques. Thus, we will discuss to what extend we are able to find correlations between certain activity patterns in the rodents olfactory bulb and verbal odor descriptors (taken from the Sigma-Aldrich Flavor and Fragrances Catalogue). Using linear classifiers (SVM with linear kernel functions), we derived predictive patterns (so-called decision images) for various human odor descriptors as well as for various odorant chemical classes. Based on these patterns, we are now able to extract useful information both on chemical classes and human odor descriptors.

With the proposed approach, several intriguing aspects can be addressed: First of all, we will discuss how similar organized is the olfactory system of human and rodents, judged by the correlation between odor quality descriptions and the rodents' bulbar activity. Furthermore, we find that the most helpful glomeruli to predict chemical classes are systematically spatially segregated from those glomeruli that carry most information to predict odor quality classes. Finally, we will give some hints on how odor quality might be spatially organized and how it might become measureable in future approaches.

Poster session I Poster #247

Olfactory perception space analysis: significant dimensions beyond pleasantness

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Recent studies based on both the analysis of odor descriptors and psychophysical results have suggested that olfactory perception is mainly dominated by odor pleasantness. While it is clear that the perceived pleasantness of an odor is likely to be an important general behavioral determinant for animals, more subtle features of odor perception clearly also play an important role in determining an animal's response to an odor.

To search for other metrics organizing odor perception space, we projected a database of descriptors used to define human odor quality (171 descriptors, describing 851 chemicals adapted from Aldrich Flavor and Fragrances Catalog) onto the nearest high dimensional Euclidean space using multidimensional scaling. The results yielded an independent and complex Euclidean interpretation of odor perception (i.e. verbal descriptors of odor quality) with approximately 32 dimensions. While analysis of this space reveals the expected organizational principle segregating pleasant from unpleasant odor descriptors, it also identifies three types of odor families, one corresponding to descriptors elicited by molecules containing nitrogen, one of molecules containing sulfur, and a third in which neither nitrogen nor sulfur are present.

Because the nutrient cycles of terrestrial ecosystems are also built around nitrogen, sulfur, and carbon, these results suggest that beyond a basic metric of pleasant and unpleasantness, human olfactory perception also reflects an olfactory stimulus space organized around naturally occurring biogenetic pathways. This in turn suggests that the human olfactory system likely has an intrinsic metric reflecting the metabolic structure of the natural world, with important implications not only for human perception but also for the organizational structure of the human olfactory system, from its receptors to the neural circuitry underlying odor recognition.

Poster session I Poster #47

Putative region for connection between the olfactory and the gustatory neurons for feeding behavior facilitation in the blowfly, *Phormia regina*

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Olfaction and taste are different modalities of chemical sense, but both information are integrated and influence feeding behavior in animals. For nectar-sucking insects like Phormia regina, floral scents repeatedly experienced with the sweet taste of nectar are recognized as preferable signs of foods, and thus the odors inform them much about foods. The blowfly, P. regina, prefers the sucrose solution flavored by 1-Octen-3-ol to plane sucrose. When we abladed the maxillary palps (accessory olfactory organ), blowflies did not show such an odor-dependent feeding preference with 1-Octen-3-ol, although when we abladed the antennae (main olfactory organ), they preserved it. This means that maxillary palps rather than antennae are inputs for this "appetitive odor". On the maxillary palps, olfactory sensilla are concentrated in the distal area. We ablated those sensilla and introduced a fluorescent dye into the olfactory neurons, thus unexpectedly found that the olfactory neurons from the maxillary palps projected to the subesophargeal ganglion (SEG), the primary gustatory center in the fly brain, rather than the antennal lobe that is well known as the primary olfactory center. Next, we cut single LL (Largest) type on labellar taste sensillum and performed anterograde staining of its gustatory neurons projecting to SEG. Thus, the olfactory neurons from the maxillary palps and the gustatory neurons from the labellum share their projective area in SEG. In the present paper, we show precise images of these neuronal projections and consider a possibility that the appetitive information of 1-Octen-3-ol odor is integrated with the sweet taste information of sucrose in this region and facilitates feeding behavior of the fly.

Poster session I Poster #93

Antennal enzymes in a moth and in the fruit fly: role in odorant reception and odorant clearance.

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The analysis of the antennal transcriptome of the moth *Spodoptera littoralis* has recently revealed that a large number of detoxication enzymes belonging to various multigene families, such as carboxylesterases, cytochromes P450, UDP-glycosyl-transferases (UGT) or Glutathione-S-transferases (GST), are expressed in the olfactory organs (1).

The expression patterns of these genes and their structural diversity were analyzed. Functional tests *in vitro* with recombinant enzymes allowed us to identify two antennal specific carboxylesterases, which are able to differentially degrade volatile acetates – pheromone components or plant compounds – into the corresponding alcohols (2, 3). Here we focussed on the *in vivo* characterization of a carboxylesterase expressed in antennae of the fruit fly, and is known to degrade the volatile pheromone cis-vaccenyl acetate *in vitro*. Electrophysiological and behavioural approaches using several mutant and RNAi strains revealed that this enzyme plays a role in the physiological and behavioural dynamics of pheromone response in *Drosophila* males.

Preliminary results in *S. littoralis* also suggested that the alcohol produced by pheromone hydrolysis within the sensillum lymph is subsequently conjugated by antennal intracellular UGTs, leading to a more hydrophilic product that could be easily excreted outside the antennae. These results strongly suggest an involvement of antennal enzymes in odorant inactivation and clearance.

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Contributed talks IV "Olfactory receptors, ligand interactions and transduction mechanisms" Monday 25 June

Sequencing the olfactory receptor repertoire

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Humans have approximately 400 functional olfactory receptors, but among this set there are a large number of variations between individuals. In some cases, these variations cause a receptor to be nonfunctional in a subset of the population. These variations likely underlie inter-individual variation in olfactory perception. We identified ligands for 19 odorant receptors and, along with previously published ligands for 9 additional receptors, characterized polymorphic variation in an ethnically diverse population. We related the genotypic variation to functional variation using a heterologous system. In our population, 86% of the odorant receptors we examined had polymorphisms that caused functional changes. On average, two individuals differ functionally at 42% of their odorant receptor alleles. This high level of functional variability in the primary receptors transducing olfactory information is strongly suggestive of high interindividual variability in odorant detection at the receptor level. Here we set out to translate this variability at the receptor level to a human subject by applying a targeted capture method to enrich the odorant receptor repertoire for next-generation sequencing. We demonstrate that we are able to fully sequence an individual's OR repertoire with enough coverage to call heterozygous sites. This method can be used to identify the functional role of a single odorant receptor in olfactory perception.

Poster session II Poster #48

Differential responses to two kairomonal cues in *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes.

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Culex quinquefasciatus, Aedes aegypti, and *Anopheles gambiae* are vectors of diseases that are among the main causes of human mortality and morbidity worldwide. Host-seeking in these species is primarily regulated by olfactory cues. The most important cue for the activation of host-seeking is carbon dioxide (CO_2), the principal by-product of respiration. All three mosquito species were able to detect and follow pulsed stimuli of CO_2 at the level of olfactory receptor neurons (ORNs) housed within capitate pegs on the maxillary palps. The temporal coding capacity of *C. quinquefasciatus* CO_2-sensitive ORNs, however, was significantly lower than that of the other two species. This differential physiological response was reflected in the behavioral response to CO_2, and correlates with the CO_2 emissions from the preferred hosts for each of these species. Following activation, what other olfactory cues do mosquitoes use in host selection? Aeration extracts taken from preferred hosts were analyzed by gas chromatography coupled single sensillum recording (GC-SSR) of the capitate pegs. We identified (*R*)-1-octen-3-ol, a component in human headspace volatiles, as a physiologically active in each species, although with different sensitivities. It is interesting to note that (*R*)-1-octen-3-ol was absent from bird aeration extracts. Landing bioassays using the host aeration extracts revealed behavioral responses of the three species consistent with their host selection preferences. The addition of biologically relevant concentrations of (*R*)-1- octen-3-ol to bird aeration extracts either inhibited or increased the behavioral response of the mosquitoes, consistent with its role as a non-host and host volatile, respectively. Through electrophysiological, chemical and behavioral analyses we show that the host-seeking behavior of mosquitoes may be differentially regulated by olfactory signals emitted by potential hosts in their environment.

Poster session I Poster #371

Intracellular inhibition by some sweet and bitter amphipathic tastants of β2-adrenergic receptor (β2AR) desensitization.

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We have previously found that some membrane-permeant (amphipathic) sweet and bitter tastants can inhibit the activity of purified forms of G-protein-coupled receptor (GPCR) kinases (GRK2 and GRK5) and protein kinase A (PKA) *in vitro*. The objective of the present study was to test whether such tastants can interfere with GPCR desensitization in intact cells by their effects on receptor signaling, receptor phosphorylation and receptor internalization. Here we show that preincubation of β2AR-transfected HeLa cells with each of six membrane-permeant tastants, three bitter (naringin, quinine and caffeine) and three non-sugar sweeteners (neohesperidin dihydrochalcone, saccharin and D-tryptophan) enhanced the subsequent isoproterenol (ISO) -stimulated cAMP formation in cells by up to 2-fold. This enhancement was dependent on both the preincubation duration time and tastant concentration. Without ISO stimulation, membrane-permeant tastants had no effect on cAMP formation, and preincubation with membrane-impermeable tastants did not enhance the ISO stimulation of β2AR activity. GRK2 silencing was sufficient to completely abolish the amphipathic tastants-enhancing ISO stimulation of cAMP formation. Furthermore, the preincubation with the amphipathic tastants significantly delayed the ISO-induced GRK-mediated phosphorylation sites (serine 355-356) of β2ARs as well as β2AR internalization. Thus, membrane-permeant tastants, which are found in common food constituents, may inhibit signal-termination-related kinases intracellularly to delay β2AR desensitization *ex vivo*, and this is likely to be relevant to the desensitization of other GPCRs.

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Delwart Contributed Symposium - Higher olfactory processing Tuesday 26 June

Neural representation of odors in absence of stimuli: evidence for mental imagery in mice

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Sensory mental imagery in humans is a cognitive activity akin to perception in the absence of external stimulation, a product of sensory information retrieved from memory. The neural substrate of sensory stimulus imagination includes the activation of sensory cortices in the visual, auditory and olfactory systems. However, to what extent imagination-induced activations of sensory cortex are exact representations of the stimulus remains unknown. To address this question, we developed an animal model in which mice were able to generate sensory representations in the absence of a sensory stimulus and we found that this representation was similar to the one evoked by the stimulus. We allowed mice to associate an odorant with a visual context by exposing them to these two stimuli on a daily basis for 10 days. Subsequently, the visual context alone triggered a behavioral investigation response, as well as a neural representation in the olfactory bulb which showed significant similarities to that evoked by the olfactory stimulus and was dependent on feedback connections to the olfactory bulb. In support of this finding, a well-described computational model of the olfactory system showed that known features of the system can produce the observed results. These findings provide the evidence in an animal model of neural activation of sensory cortex during memory retrieval, reminiscent of mental imagery in humans.

Poster session I Poster #49

NaCl perception in *Drosophila* adult

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The sense of taste plays a key role in the search for food or sexual partners. The gustatory system is involved in avoidance of chemicals such as high sodium chloride (NaCl) concentration, bitter compounds, …. In adults, taste bristles (sensilla) are located on the labial palps, internal mouthpart organs, legs, wings and ovipositor. Three types of labellum sensilla have been characterized based on their morphology and eletrophysiology : small (s) and long (l) bristles are composed of one gustatory receptor neuron (GRN) that responds to water (W cell), one to sugars (S cell), one to low NaCl (L1 cell) and one to high NaCl concentrations (L2 cell), whereas intermediate (i) ones house two GRNs detecting either sugar and low salt (S cell), or bitter compounds and high NaCl (L2 cell). In legs, only one type of sensilla contains three GRNs detecting water (W cell), sugar (S cell) and salt (L2 cell), respectively. Mechanisms involved in NaCl perception are poorly understood. Several members of the DEG/ENaC channels family named *pickpocket* genes have been characterized in *Drosophila*. They are involved in the detection of both low and high NaCl concentrations. Moreover, we identified the *serrano* gene that is specifically required for high NaCl aversive response in larvae. In order to confirm its gustatory role in adults, we performed behavioural tests in two different assay conditions where different parts of the flies are in contact with the testing NaCl solution. Surprisingly, we observed either an aversive or a defective response for the *serrano* mutant. These results indicate that the detection of NaCl by the L2 cells of the labellum sensilla and the L2 cells of the leg bristles are mediated by two different cellular mechanisms. Our study showed that Serrano function is only required in L2 cells of labellum sensilla for the detection of high NaCl.

Poster session II Poster #50

Evolution of olfactory preference in *Drosophila suzukii* (Diptera:Drosophilidae)

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The spotted wing *Drosophila* (SWD), *Drosophila suzukii* Matsumura**,** is an invasive pest of soft fruit crops and becoming an economic threat in Europe. This species has a different behavior ecology from most other drosophilids, as the female lays eggs on healthy and ripening fruits, while most members of its family select overripe or decaying fruits. Using a variety of tools we are analyzing if and how this niche differentiation has led to changes in the layout and function of the olfactory circuitry of adult *D. suzukii* compared to *D. melanogaster*. By using immunohistochemistry we are comparing the antennal lobes of *D.suzukii* and *D.melanogaster.* Changes may be reflective of ecological adaptations of adult *D.suzuki* to prefer fresh fruits.

Symposium 20 "Aquatic olfaction" Tuesday 26 June

Subsystem organization of an amphibian nose

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Amphibians represent an evolutionary intermediary between fish and terrestrial species. Accordingly, their olfactory organs have elements of both, aquatic as well as terrestrial vertebrates. Most amphibians, including *Xenopus laevis*, have a main as well as an accessory (vomeronasal) olfactory system. The main olfactory system of larval *Xenopus laevis* is composed of at least two subsets of olfactory receptor neurons. Here we show that these subsets together with their bulbar target regions constitute two almost completely independent olfactory subsystems. Their olfactory receptor neurons in the olfactory epithelium as well as their glomeruli in the olfactory bulb are almost completely segregated (lateral vs. medial location). They have clearly diverging odorant sensitivities, distinct transduction mechanism with differing G-proteins, olfactory receptor neurons with different morphology (microvillous vs. ciliated), and differences in the expression frequencies of olfactory receptor genes. Based on these properties, the coding logic of the *Xenopus* olfactory system appears to be a true intermediate between the olfactory system of fish and higher tetrapods. The single sensory surface of fish shows little segregation, whereas the mammalian olfactory system is known for extensive regionalization of olfactory subsystems. Here we show that the amphibian olfactory system shows distinct, but limited regionalization and appears thus to be well-suited to investigate the molecular driving forces behind olfactory regionalization.

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Symposium 16 "Taste and beyond - integration of nutrient sensor functions in oral cavity and gut - Ajinomoto Symposium" Tuesday 26 June

Taste cells of the gut and endocrine cells of the tongue

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We have found that many of the receptors and downstream signalling elements involved in taste detection and transduction are expressed also in intestinal hormone producing (endocrine) cells where they underlie key chemosensory functions of the gut. In one example of gastrointestinal chemosensation it is known that glucose given orally, but not

systemically, induces secretion of the "incretin" hormone GLP-1 (glucagon like peptide-1), which in turn regulates insulin secretion and glucose homeostasis. We have found that intestinal endocrine cells express sweet taste receptors, the taste G-protein gustducin, and several other taste transduction elements. Knockout mice lacking gustducin or the sweet taste receptor subunit T1R3 have deficiencies in secretion of GLP-1 and in the regulation of plasma levels of insulin and glucose. We have studied intestinal cell lines that express gustducin, taste receptors and other taste signaling elements to identify the roles of these taste proteins in regulating GLP-1 hormone release. In another example of gastrointestinal chemosensation we have found that endocrine cells of the pancreas express multiple taste proteins that are involved in regulating insulin release. Furthermore, taste cells of the oral cavity express GLP-1, other "gut" hormones and the insulin receptor. Most recently, we have identified intestinal-type glucose transporters and pancreatic-type ATP-gated K+ channels (K-ATP metabolic sensors) as being present in taste cells and potentially functioning in the detection of the sweet taste of sugars. In sum these studies point out similarities in gustation and gut chemosensation and indicate the importance of "taste cells of the gut" and "endocrine cells of the tongue" in coordinating the body's hormone responses to regulate glucose homeostasis. Supported by NIH grants DC03055, DC03155 and DK081421.

Symposium 21 "Molecular and neural basis of taste detection" Wednesday 27 June

Bitter-modulated sugar detection provides an independent way to avoid ingesting noxious molecules

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Sensory cells detecting "bitter" taste are usually considered as essential to avoid potentially toxic chemicals. To test this assumption, we used a genetic approach to deprive flies from their bitter-sensitive cells. Unexpectedly, these flies were still able to avoid ingesting sugar solutions contaminated with alkaloids like quinine and strychnine, in the context of binary or multiple choice feeding tests, or when monitoring their proboscis extension response. Sugar-sensing cells are directly inhibited by alkaloids even in the absence of a neighboring bitter-sensitive cell. Interestingly, some chemicals which activate bitter-sensing cells like caffeine and L-canavanine, have almost no effect on inhibiting the response to sucrose, indicating that bitter-sensing gustatory receptors and sugar-sensing inhibition occur through different transduction pathways. Avoiding toxic molecules involves thus at least two distinct systems which may have evolved independently: (1) bitter-sensing cells excited by bitter molecules triggering active avoidance reactions, and (2) sugar-sensing cells silenced by noxious molecules, making food less palatable.

Poster session I Poster #267

Identification of gustatory-olfactory flavor mixtures: Effect of linguistic labeling

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In this study, we examined the effects of verbal labels on the identification of flavor mixtures (aqueous solutions) containing various proportions of sucrose (gustatory flavorant) and citral (olfactory flavorant). For each subject, we first determined concentrations of sucrose and citral perceived as equally intense. Then, again for each subject, we used these matching concentrations to construct four solutions containing different proportions of the two flavorants: 35% sucrose/65% citral, 45% sucrose/55% citral, 55% sucrose/45% citral, and 65% sucrose/35% citral. In the 80 trials of each session of the main experiment, we randomly presented all 4 mixtures a total of 20 times each, asking the subjects, on each trial, to identify the flavor stimulus as mostly 'sugar' or mostly 'citrus.' In the experimental condition (12 subjects), each flavor stimulus was preceded by a verbal label, either "SUGAR" or "CITRUS," which, the subjects were informed, was sometimes but not always the name of the greater flavor component. That is, the labels were probabilistically valid. In the control condition (another 12 subjects), the labels were omitted. The results showed that the labels systematically modified the identification responses compared to the no-label, control condition. For each of the four mixtures, presenting the label 'SUGAR' increased the responses of 'sugar', whereas presenting the label 'CITRUS' increased the

responses of 'citrus'. We compare the results to predictions of optimal and partial integration of linguistic and sensory information.

Poster session II Poster #334

CaSR agonist enhances sweet taste signal via cell-to-cell communication in taste buds

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Recently, we reported that agonists of the calcium-sensing receptor (CaSR), including γ-glutamyl-cysteinyl-glycine (glutathione) and other γ-glutamyl peptides, enhance specific basic tastes—umami, sweet, and salty. This enhancement is called "*kokumi*" flavor. Interestingly, CaSR agonists enhance specific basic tastes but do not elicit any taste themselves. In this study, we identified the receptor cells for the CaSR agonists and characterized how these enhancers affected basic taste signals. In the lingual slice preparation, applying the taste enhancer γ-glutamyl-valinyl-glycine focally at the taste pore induced intracellular Ca^{2+} responses in a subset of taste cells. These responses were strongly inhibited with the CaSR antagonist, NPS-2143. Anti-CaSR immunopositive taste cells were observed in circumvallate, foliate, fungiform and palate taste buds. Surprisingly, these taste cells comprised a totally different subset than T1R3-positive taste cells. Using ATP biosensors, we found that the artificial sweetener SC45647 triggered ATP secretion from isolated mouse circumvallate taste buds and that ATP secretion was enhanced by co-applying γ-glutamyl-valinyl-glycine. This enhancement was abolished by atropine, an inhibitor of muscarinic acetylcholine receptors. Almost all CaSR positive taste cells co-expressed acetylcholine markers, indicating that CaSR positive taste cells contain acetylcholine. These observations indicate that *kokumi* flavor stimulation enhances sweet taste-induced ATP release from sweet receptor cells via cholinergic cell-to-cell (paracrine) signaling within a taste bud (Dando *et al*. AChemsS 2010).

Poster session II Poster #268

Association of odor-induced autobiographical memories with slow breathing and amygdala-hippocampal activity

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An important feature of olfactory perception that has been relatively neglected is its dependence on respiratory activity. Inspiration is related to the perception and recognition of emotions, and can trigger memory retrieval through olfactory sensations.

We previously analyzed electroencephalograms (EEGs) and respiration simultaneously in normal subjects while testing for detection and recognition of odors. We assumed that if perception of an odor and related emotions depend on inspiration, we would find that the averaged inspiration-related potential during presentation of olfactory stimuli is triggered by inspiration onset. We found that 9–12 Hz cortical rhythms were phase-locked to inspiration during odor stimulation in a process referred to as inspiration phase-locked alpha band oscillation (I-α)(Masaoka et al., 2005). The genesis of I-α has been identified in olfactory-related areas in the brain, including the entorhinal cortex (ENT), hippocampus (HI), amygdala (AMG), and orbitofrontal cortex (OFC), using an EEG dipole tracing method. It is well known that some odors elicit special emotion or memory retrieval. Memories induced by odors enable individuals to mentally travel back into their personal past. These are episodic or autobiographical memories. In this study, using the same technique previously reported, we investigated how brain areas during stimulation of odor associated with personal memory differ from those activated by control odors. During presentation of odors related to autobiographical memories and control odors, EEG and respiration were simultaneously recorded. We found that odor-induced autobiographical memory retrieval was associated with increasing tidal volume and decreasing respiratory frequency more than during presentation of control odors. In addition, we found that odors associated with autobiographical memory activated the

ENT, HI, and AMG to a greater extent than did control odors. These activations were observed from 50 ms to 100 ms after the onset of inspiration. We discuss a relationship between slow breathing and memory consolidation related to the mechanisms of brain rhythm and respiration.

Poster session I Poster #117

Olfactory neural circuitry mediating Schreckstoff-evoked alarm response in zebrafish.

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Various fish species including zebrafish respond to a putative alarm pheromone (Schreckstoff) released from injured skin of conspecifics and display robust aversive behavior. However, molecular, cellular, and neural circuit mechanisms underlying the alarm response remain largely unknown. In this study, we first developed a semi-automatic behavioral analysis system of the zebrafish alarm response based on swimming velocity, cumulative distance, and location in the tank. Upon application of conspecific skin extract, most zebrafish showed a biphasic response: initial burst swimming followed by freezing at the bottom of the tank. To identify peripheral and central olfactory neurons activated by skin extract, we immunohistochemically examined phosphorylation of Erk (MAP kinase), a reliable maker for neuronal activation in zebrafish. In the skin extract-stimulated olfactory epithelium, phospholylated Erk (pErk) immunoreactivity was observed in a subpopulation of olfactory sensory neurons (OSNs) with heterogeneous morphology, including superficially located ovoid OSNs, and bipolar OSNs with long and short dendrites. In the olfactory bulb, pErk immunoreactivity was detected in three discrete glomeruli. We further observed pErk-positive neurons in restricted regions of the telencephalon, preoptic area, and hypothalamus of zebrafish that showed the alarm response. Our study provides a neuroanatomical basis for understanding the neural circuit mechanisms for Schreckstoff-evoked alarm response in zebrafish.

Poster session I Poster #335

Dried-bonito flavoring enhance salivary hemodynamic responses to broth tastes detected by near-infrared spectroscopy

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Dried-bonito (*Katsuo-bushi*) broth, an important seasoning in Japanese cuisine, has long been used to reinforce the flavor of appetitive foods. Not only the taste but also the aroma components of the broth contribute well to the enhancement. To elucidate the effects of aroma from dried bonito on broth tastes caused by the central integration of flavor, we performed optical imaging of salivary hemodynamic responses using near-infrared spectroscopy (NIRS). The recording with NIRS of salivary hemodynamic signals, which have been reported to be accompanied by saliva secretion from the parotid gland in response to taste stimuli, enabled us to test panelists in a normal, seated position with minimal restriction of movement during drinking.When hemodynamic responses to a reconstituted dried-bonito flavored broth were compared to those of odorless broth taste solutions, the flavored broth produced significantly larger responses than the odorless broth. This was the case for five of the ten panelists, who felt that the combination of the aroma with the tastes was congruent. In the remaining five who felt the combination incongruent, the flavored broth did not cause the enhancement of responses. Moreover, when (4*Z*, 7*Z*)-4,7-tridecadienal, one of the most important key aroma components of dried-bonito extracts, was added to the flavoring, the latter five as well as the former five participants came to feel congruency of the reconstituted aroma with broth tastes. The reconstituted flavored broth produced significantly larger hemodynamic responses than the odorless broth in both participants' groups. Accordingly, there is a positive correlation between the degree of congruency and increase of the salivary hemodynamic responses. These results indicate that NIRS offers a sensitive method to detect the effect of flavoring on the taste-related salivary hemodynamic responses, dependent on the perceptual experience of the combination of aromas and tastes.

Poster session I Poster #51

Oviposition behavior and preference in the mosquito *Aedes aegypti*

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Oviposition (egg-laying) is a critical point in the mosquito's life cycle. Larval and pupal mosquitoes are aquatic. Thus, a female mosquito must find a suitable body of water in which to lay her eggs. We study this behavior in the yellow and dengue fever mosquito *Aedes aegypti* with the goal of understanding how the female detects the presence of liquid water and the sensory information that influences the decision of where to lay her eggs.

Our behavioral studies indicate that females utilize both volatile and contact sensory cues during egg-laying. Prior to an egg-laying bout, a female's legs, ovipositor, and/or proboscis will contact the surface of the water and the moist substrate above the waterline. We are using an Illumina RNA-sequencing approach (RNA-seq) to build a comprehensive profile of transcripts expressed in these tissues, with a focus on those with female-specific expression or that are regulated by the feeding state of the animal. This list, which includes genes from the three chemosensory receptor families, will provide candidate genes involved in the peripheral sensation of water and water quality and thus potential targets for mutagenesis and neural circuit analysis.

We have also begun an artificial selection experiment to examine the genetic mechanisms underlying oviposition site preference. Some populations of *Aedes aegypti* have specialized on human hosts and have concurrently gained an increased tolerance for laying eggs in clean water, such as storage vessels associated with human dwellings. By selecting for females that tend to lay their eggs in clean vs. soil-infused water cups over multiple generations, we aim to generate behaviorally divergent strains. Comparing these strains through tissue-specific RNA-seq will allow us to identify candidate genetic elements controlling preference. Together, we hope that these approaches will help us further understand mosquito oviposition with potential implications for vector control.

Contributed talks VI "Interactions" Monday 25 June

Genetic analysis of olfactory preference for humans in evolutionarily divergent forms of the Dengue Fever Mosquito, *Aedes aegypti*

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Many organisms rely on innate olfactory preferences to find food and avoid danger. Several decades of elegant work in model systems such as *Drosophila* and mouse have described the peripheral genes and neurons that mediate olfaction. However, we still know very little about how innate olfactory preferences are genetically encoded. To address this issue, we have taken advantage of natural variation between two forms of the Dengue Fever Mosquito, *Aedes aegypti*. An ancestral, forest form prefers the odor of non-human animals, while a more recently evolved, domestic form strongly prefers human odor. Classic work from the 1970's and 1980's showed that forest and domestic forms coexist in several places along the coast of East Africa, where they maintain their ecological differences despite being fully interfertile. This situation provides an unusual opportunity to examine the genetic basis of olfactory preference. We have verified the continued coexistence of human and animal-adapted mosquito populations in the Rabai region of Kenya, established laboratory colonies, and documented striking, genetically-based differences in preference for human *vs*. animal scent. Using high-throughput transcriptome sequencing of tissues involved in the reception and processing of odor cues (antenna, maxillary palp, brain), we have also identified several genes whose expression and/or sequence differs between forest and domestic mosquitoes and is associated with preference in a large F2 hybrid population. Ongoing work aims to test the role of specific candidate genes on preference.

Poster session II Poster #336

The enhancement of flavor detection under conditions of disgust.

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Current conceptualizations of disgust describe its emotive components and behavioral consequences, yet little research has examined the sensory impact of disgust. As a food-related emotion with broad behavioral significance, disgust should be expected to exert an influence on chemosensation, but to date no such influence has been reported. Three experiments are described that examined the impact of disgust on flavor detection. In each experiment participants read stories with descriptions of disgust-inducing stimuli, while those in control conditions read non-disgusting versions of the stimuli. Following this mood induction, a flavor detection task was used to assess participants' thresholds for detecting the presence of a target flavor in a base solution to determine whether disgusted participants' thresholds differed from non-disgusted participants. Thresholds were assessed using a 2AFC with reference procedure, and concentrations were adjusted on each trial following a 2-down, 1-up staircase method. The three experiments examined the impact of different categories of disgust elicitors on flavor detection. In experiment 1, participants who read "core" disgust, food-related stories had lower thresholds (higher sensitivity) to the target flavor. In experiment 2, "core" disgust elicitors that were not food-related similarly enhanced flavor sensitivity. In experiment 3, "animal-reminder" (i.e.,contact with death) disgust elicitors did not impact sensitivity, while a comparable animal-related core disgust elicitor did. [all ps < .05]. Together the studies point to a top-down role for disgust in modifying flavor sensitivity, and suggest that conceptualizations of disgust need to be extended to include its sensory consequences.

Poster session I Poster #213

A cell-centric olfactory gene expression atlas

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Gene expression atlases of tissues are useful tools but none yet allow the compilation of lists of all genes expressed by specific cell types and therefore have limited utility for understanding cells as functional units. We have developed a novel strategy to identify all genes expressed in specific cell types. Enrichment ratios for each mRNA in cell types isolated by flow cytometry are calculated, validated using an in situ hybridization database, and then categorization models generate probabilities of expression for each gene in each cell type in the tissue. These probabilities were 96% accurate at identifying genes expressed in olfactory sensory neurons (OSNs) and 86% accurate at discriminating genes specific to mature or immature OSNs. The data identify 14,287 genes expressed in the olfactory epithelium, 8,299 of them predominantly in OSNs with 847 and 691 genes (excluding odorant receptors) specific to immature and mature OSNs, respectively. Bioinformatics analyses of known genes identified biological processes and functions over-represented in these cell types. Control of gene expression by chromatin modification and transcription factors, along with neurite growth, protein transport, RNA processing, cholesterol biosynthesis, and apoptosis via death domain receptors were overrepresented in immature OSNs. Ion transport (ion channels), presynaptic functions, and cilia-specific processes were overrepresented in mature OSNs. Processes overrepresented among the genes expressed by all OSNs were protein and ion transport, ER overload response, protein catabolism, and the electron transport chain. In addition, transcripts from thousands of genes encoding proteins of unknown function were identified, thereby predicting that many of them contribute to these known aspects of OSN phenotypes. Files containing the gene expression atlas and the in situ hybridization database are available.

Poster session I Poster #123

Comparison of nasal air flow and impact on odor perception in several mammalian species.

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The peri-receptor events are the first stage in the olfactory perception process (Watelet et al., 2009), but the detailed mechanisms by which odorants in inspired air are transported to the olfactory receptors are not fully understood. Inspiration of odorant-containing air into the nostril, follows a tortuous route to the olfactory epithelium located high in the nasal cavity of the mammalian species studied to date. Nasal cavities have been mapped for human, dog, rat (Pereira et al., 2011), and now cat (Yee et al., 2011), and show different degrees of tortuosity. Computational fluid dynamics (CFD) has been used by several research groups (e.g. Craven et al., 2010; Schroeter et al., 2006; Zhao et al., 2006) to mathematically model air flow in these nasal passages. We review the similarities and differences in air flow between the species especially with respect to the changes that occur when "sniffing" takes place and how the increase in air flow rate affects the distribution of air through the nasal passages, as well as the predicted deposition of odors on the olfactory epithelium. The key factors considered in the CFD models are also reviewed and suggestions for further developments in mathematical modeling proposed to build models that could better predict the odor profile delivered to the olfactory receptors during resting breathing and sniffing.

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Symposium 17 "Toward a genetic basis for human olfaction" Tuesday 26 June

Therapeutic gene rescue of an olfactory ciliopathy

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Olfactory dysfunction is now recognized as a clinical manifestation of a broad class of human diseases, termed ciliopathies. Cilia on olfactory sensory neurons (OSNs) are evolutionarily conserved microtubule-based organelles in which all the components necessary for odor detection are compartmentalized. Disruption of cilia formation or protein localization to cilia results in olfactory deficits. Within the past 5 years patients with genetic mutations resulting in olfactory ciliary defects have been clearly identified in 2 different pleiotropic diseases, Bardet–Biedl Syndrome (BBS) and Leber congenital amaurosis (LCA). Despite significant progress identifying the genes underlying ciliopathies, curative therapies are not yet available to patients. We demonstrate that mice with a hypomorphic mutation in the intraflagellar protein, IFT88 (ORPK, $ift88^{Tg737Rpw}$) are functionally anosmic, due to the loss of cilia on OSNs. Importantly, viral-mediated re-expression of IFT88 in differentiated OSNs is sufficient to restore cilia structures and rescue olfactory function. These studies represent the first *in vivo* therapeutic treatment to reestablish cilia in a mammalian ciliopathy, and show that gene therapy is a viable curative option for functional rescue of the complex cilia organelle in established differentiated cells.

Poster session II Poster #214

Post-translational modifications regulating ciliary localization of olfactory signaling proteins

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In olfactory sensory neurons (OSNs), the protein components necessary for odor detection are highly enriched in cilia. However the precise mechanisms for this localization remain poorly defined. Recent work indicates that mechanisms for selective cilia entry may be analogous to nuclear import utilizing both importin b2 and a Ran gradient. A unique post-translational modification process that is involved in nuclear-cytosolic transport, but has not been examined in ciliary localization is the reversible conjugation of Small Ubiquitin-like Modifier (SUMO) proteins or SUMOylation. Bioinformatic examination of several olfactory signaling protein sequences revealed that both adenylate cyclase 3 (AC3) and the calcium-activated chloride channel, annoctamin2 (ANO2) harbor conserved SUMOylation motifs. Therefore, we hypothesized that SUMO modification regulates ciliary localization of AC3 and ANO2. Coexpression of the SUMO protease SENP2 with either AC3:GFP or ANO2:GFP in MDCKII cells blocked their normal ciliary localization, indicating a role for SUMOylation in ciliary trafficking. In addition, site directed mutagenesis of the predicted SUMOylation sites abolished ciliary localization of both proteins. Live cell, *en face* imaging of adenovirally infected OSNs shows that wildtype AC3:GFP traffics to olfactory cilia, while the SUMO deficient form is retained within the dendritic knob. Finally, we tested if SUMOylation is sufficient for ciliary entry, by exploiting the distinct properties of the related ANO1 and ANO2 proteins. Reconstitution of consensus SUMOylation motifs in ANO1 through mutagenesis was not sufficient to convert ANO1 from a cilia-excluded protein into a cilia-targeted one. Together our data demonstrate that SUMOylation of olfactory signaling proteins is necessary, but not sufficient for ciliary localization in OSNs, strengthening the mechanistic link between nuclear and cilia trafficking.

Poster session II Poster #52

Genes and neural circuits controlling sensitivity to carbon dioxide in the mosquito

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Blood-feeding mosquitoes, such as the yellow fever mosquito *Aedes aegypti*, use highly specialized and sensitive sensory systems to locate their hosts. This sensory process involves detecting and following plumes of volatile host emissions, which include skin odor and carbon dioxide (CO_2). In mosquitoes, behavioral evidence suggests that CO_2 functions as a powerful attractant and additionally acts to sensitize the mosquito olfactory system, alerting them to the presence of human skin odor and heat. Despite the critical importance of CO_2 reception in driving the orientation of mosquitoes towards humans, the molecular and cellular basis of how this gas is sensed and integrated with other host chemical and physical cues to initiate host-seeking behavior is poorly understood. As a first step toward dissecting CO_2 sensation in the mosquito, we used zinc-finger nuclease (ZFN) technology to generate null mutations in two gustatory receptors, *AaGr1* and *AaGr3*, which are candidate CO_2 receptors in *A. aegypti*. We further characterized the electrophysiological and behavioral responses of these mutant mosquitoes to CO_2. Nonsense mutations were successfully recovered for both *AaGr1* and *AaGr3* using ZFN-mediated targeted mutagenesis. *AaGr3* null mutants were selected for initial characterization, and were found to be electrophysiologically unresponsive to CO_2. Furthermore, in behavioral paradigms of CO_2-induced heat seeking and live host feeding, *AaGr3* mutants were behaviorally insensitive to CO_2, when compared to wild-type mosquitoes. Our data provide compelling evidence that *AaGr3* is required for CO_2 sensation in *A. aegypti*, and suggests that CO_2 sensation gates the response of female mosquitoes to heat. Further evidence for the redundant integration of other sensory cues for host-seeking, and the spatial scale over which CO_2 sensation acts to elicit mosquito behavior will be presented.

Poster session I Poster #53

Plasticity in the antennal expression of amine-receptor genes in the honey bee *Apis mellifera* L.

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A number of lines of evidence suggest that in insects, behavioural responses to olfactory signals may be regulated via the peripheral actions of biogenic amines. Recently we demonstrated that changes in biogenic amine receptor gene expression in the antennae correlated with shifts in the behavioural responsiveness of worker honey bees to queen mandibular pheromone, a response also known to vary with age. Here we used behavioural manipulation to examine more broadly whether gene expression in the antennae of the bee changes with age and behavioural state. Single- and double-cohort colonies were used to generate nurses and foragers of the same age. We found that expression of the octopamine receptor gene, *Amoa1*, was strongly affected by behavioural state. In young bees (6- and 15-day olds) *Amoa1* expression was lower in foragers than in nurses of the same age. The tyramine receptor gene, *Amtyr1*, showed the opposite trend. Of the genes examined in the study, dopamine receptor gene transcripts appeared to be least affected by the induction of precocious foraging, although *Amdop1* expression levels were found to be significantly lower in 6-day old nurses than in foragers of the same age. These results confirm that the regulation of amine-receptor gene expression in the antennae is highly dynamic and may contribute to the behavioural plasticity so characteristic of this highly social insect.

Poster session I Poster #361

Overweight/obese children have altered glutamate taste perception.

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Obese women have significantly higher thresholds for monosodium glutamate (MSG), but not for other tastes (1). This study addressed the relationship between weight, and taste threshold and liking in children (<18 years).

Approval for the study was given by the relevant local Research Ethics Committees. Sixty nine children gave consent/assent;seven were subsequently excluded (age >18 on testing or incomplete data sets). Participants (n=62, age range 5-17 years) were weighed, and height measured and BMI centile-for-age calculated (range 4 - >97th centile). Taste thresholds for salt (NaCl), sweet (sucrose) and glutamate (MSG) were determined using a 2-alternative forced-choice staircase method. Liking for each taste was determined from a single presentation of 100mM NaCl, MSG and sucrose and rated using the "Faces Scale" (2).

Salt and MSG discrimination thresholds were significantly correlated with BMI centile, whereas there was no association between sucrose threshold and weight. There were no differences in taste thresholds in boys, but overweight/obese girls (>85th centile) had significantly higher salt and MSG taste thresholds than normal weight girls. There were no significant differences in liking of different tastes in boys and girls of different weights.

These first data on savoury taste perception in children demonstrate that, as in adults, savoury taste thresholds are altered in females with high BMI compared to males and females of normal weight. Obesity, particularly in females, seems linked to a blunting of savoury taste.

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Poster session II Poster #114

Chemoreception in the antennules of the red swamp crayfish *Procambarus clarkii*

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The antennules of crayfish, like in other decapod crustaceans, have been reported as olfactory organs involved in food searching, mating and agonistic behaviour (Fedotov, 2009). The range of stimulatory compounds may vary among species, but typically includes amino acids, nucleotides and their derivatives, amines, bile acids and, in some cases, carbohydrates.

The red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Crustacea: Decapoda), a worldwide invasive species, has biramous antennules with a lateral flagellum bearing aesthetasc chemoreceptors with a role in intraspecific communication (Horner et al., 2008) and a medial flagellum, the sensory processing of which is still unclear. In the present study we evaluate the stimulatory effectiveness, on the antennular chemoreceptors of adult *P. clarkii*, of a few amino acids and carbohydrates to which the pereopod (walking leg) chemoreceptors were previously reported to be responsive, and taurocholic acid, which instead does not stimulate pereopods (Corotto and O'Brien, 2002; Corotto et al., 2007).

To this end, the firing patterns in the nerves emerging from both the lateral and medial flagellum were recorded under chemical perfusion, by way of extracellular suction electrodes (A-M System differential amplifier).

Results show that, among the tested compounds, maltose and taurocholic acid stimulate chemoreceptor cells from both the lateral and medial flagellum of antennules in adult crayfish, with the latter stimulus being effective in males as well as in females.

Even though at present a functional significance of the antennular sensitivity to maltose and taurocholic acid cannot be assigned, the antennular vs. pereopod activity is discussed in the light of possible overlapping/complementary roles of the two sensory organs in providing the animal with the ability to locate key odours and eventually for the development of strategies for population control programmes.

Poster session II Poster #54

RNASeq identification of genes enriched in *Drosophila* coeloconic sensilla

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In *Drosophila*, olfaction relies upon olfactory receptor neurons (ORNs) housed in three morphological classes of sensory hairs: basiconic, trichoid, and coeloconic sensilla. In most insect species, coeloconic ORNs primarily respond to amines and acids, two classes of odors that are not generally detected by other ORNs and are behaviorally relevant for many insects. In *Drosophila*, coeloconic ORNs have been shown to utilize Ionotropic Receptors (IRs) as olfactory receptors rather than the canonical Odorant receptors (Ors) that mediate odor detection in other ORNs. However, the contribution of coeloconic ORNs to olfactory coding and behavior remains enigmatic. To identify genes that are highly enriched in coeloconic sensilla and that may contribute to their unique olfactory function, we undertook a high-throughput RNA sequencing screen of antennae from wild-type flies and atonal mutants, which selectively lack coeloconic sensilla. We identified approximately 400 candidate genes whose expression is eliminated or reduced in atonal antennae. These genes include the expected IRs as well as a small subset of olfactory binding proteins (OBPs). Overall, the candidate genes have diverse biological functions and are predicted to encode such varied proteins as ion channels, GPCRs, signal transduction components, and transcription factors. Approximately 1/3 of candidate genes have no predicted function. We are currently investigating the role of a neuronal ionotropic glutamate receptor that is distinct from but related to the IR family and whose expression is enriched in coeloconic sensilla. This study will guide further research into the molecular underpinnings of olfactory coding in the antenna.

Poster session I Poster #423

Expression of Tas1 taste receptors in mammalian spermatozoa: functional role of Tas1r1 in regulating basal Ca^{2+} and cAMP concentrations in spermatozoa

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During their transit through the female genital tract, sperm have to recognize and discriminate numerous chemical compounds. However, our current knowledge of the molecular identity of appropriate chemosensory receptor proteins in sperm is still rudimentary. Considering that members of the Tas1r family of taste receptors are able to discriminate between a broad diversity of hydrophilic chemosensory substances, the expression of taste receptors in mammalian spermatozoa was examined. Choosing complementary molecular, cellular and reproductive approaches we found that the two subunits of the umami taste receptor dimer (Tas1R1/Tas1R3) are expressed in mouse and human spermatozoa where their sub-cellular localization is restricted to distinct segments of the sperm flagellum and the acrosomal cap of the sperm head. Employing a Tas1r1-deficient mCherry reporter mouse strain, we found a significant increase in spontaneous acrosomal reaction in Tas1r1 null mutant sperm whereas acrosomal secretion triggered by isolated *zona pellucida* or the Ca^{2+} ionophore A23187 was not different from wild-type spermatozoa. Remarkably, cytosolic Ca^{2+} levels in freshly isolated Tas1r1-deficient sperm were significantly higher compared to wild-type cells. Moreover, a significantly higher basal cAMP concentration was detected in freshly isolated Tas1r1-deficient epididymal spermatozoa, whereas upon inhibition of phosphodiesterase or sperm capacitation, the amount of cAMP was not different between both genotypes. Since Ca^{2+} and cAMP control fundamental processes during the sequential process of fertilization, we propose that the identified taste receptors and coupled signaling cascades keep sperm in a chronically quiescent state until they arrive in the vicinity of the egg - either by constitutive receptor activity and/or by tonic receptor activation by gradients of diverse chemical compounds in different compartments of the female reproductive tract.

Symposium 9 "Chemosensory initiated mating behaviour" Sunday 24 June

The perfume of MHC immunogenes

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Sexual selection has been proposed as one mechanism to explain the maintenance of high allelic diversity in MHC (Major Histocompatibility Complex) genes that control the extent of resistance against pathogens and parasites in natural populations. MHC-based sexual selection is known to involve olfactory mechanisms in fish, mice and humans. During mate choice, females of the three-spined stickleback (*Gasterosteus aculeatus*) use an odour-based selection strategy to achieve an optimal level of MHC diversity in their offspring, equipping them with optimal resistance towards pathogens and parasites. The molecular mechanism of odour-based mate-selection strategies has been a long-standing puzzle. We have studied the nature of this signalling system and found the highly polymorphic signal being MHC peptide ligands - the natural perfume. We can synthesise them and by adding this substance can manipulate the effect of a male's own signal predictably - at least in sticklebacks. The substance is the same in mice and most probably in humans. If MHC peptide ligands are the signal also in humans they could improve the biological function of commercial perfumes.

Poster session I Poster #101

Are insect olfactory receptors an adaption to a terrestrial lifestyle?

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Insects detect volatile molecules employing olfactory receptors (ORs) present in the dendritic membrane of olfactory sensory neurons (OSNs). Recently, members of a distinct group of receptors related to ionotropic glutamate receptors (IRs) were established to function as olfactory receptors as well. The IRs are expressed in olfactory organs across Protostomia, whereas ORs of the insect type are hypothesized to be an adaption to a terrestrial lifestyle. Here we present a detailed analysis of the olfactory system of *Lepismachilis y-signata,* a member of the Archaeognatha, a very basal, wingless insect group that emerged an estimated 390 mio years ago. Using electrophysiological, morphological and molecular techniques we demonstrate that *L. y-signata* possesses an acute but reduced olfactory system, displaying a unique morphological and molecular makeup among extant insects. Using a wide range of molecular techniques, including transcriptom analysis, microarrays, as well as immunohistochemistry we were not able to detect the presence of any ORs, although IRs are clearly present. Fluorescent *in situ* hybridization allowed us to ascertain that the identified IRs are indeed expressed in OSNs. Our findings indicate that ORs did not arise as an adaption to a terrestrial lifestyle, but rather evolved later in insect evolution.

Supported by the Max-Planck-Society and the International Max-Planck Research School.

Poster session II Poster #134

Olfactory circuit enhancer (OCE) in mouse Tbx21 gene directs efficient transgene expression along the olfactory neural pathways in mice and zebrafish

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Tbx21, a T-box family transcription factor, is specifically expressed in the olfactory bulb output neurons in both mouse and zebrafish. Previously, we performed promoter/enhancer analysis of the mouse Tbx21 gene by transgenesis and identified a cis-regulatory element crucial for its mitral/tufted cell-specific expression. This ~300-bp sequence at 3.2-kb upstream of mouse Tbx21 gene was designated as MCE (mitral/tufted cell-specific enhancer). In the course of study, we noticed another cis-regulatory element (~700 bp) at 2.6-kb upstream of Tbx21 gene, which is required for transgene expression in neurons along the olfactory neural pathway, and termed it OCE (olfactory circuit enhancer). In this study, we performed detailed immunohistochemical analysis of brain sections from the OCE transgenic mouse lines and observed transgene expression predominant along the olfactory circuitry and variable from line to line (e.g. olfactory sensory neurons, OB granule cells, anterior olfactory nucleus, nucleus of lateral olfactory tract, lateral entorhinal cortex, etc.). Next, we applied this mouse OCE element for enhancer trap screening in zebrafish and successfully obtained a number of transgenic fish lines in which Gal4 was expressed with high efficiency in distinct subsets of the olfactory sensory neurons as well as in central neurons in various telencephalic regions. These results demonstrate that the OCE can function as a selective and efficient transcriptional enhancer directing transgene expression in various types of neurons along the olfactory pathway in both mice and zebrafish. Furthermore, the present study implies evolutionary conservation of a common transcriptional mechanism for neural circuit-specific gene expression in vertebrates.

Poster session II Poster #338

Shh-expressing basal cells are immediate precursors of Type I, II and III cells in taste buds

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Taste buds consist of heterologous populations of cells classified into Type I, II, III and IV, and are maintained by continuous cell renewal throughout life in mammals. Type IV cells are the basal cells in taste buds, and we previously reported that most of them express an evolutionally conserved signaling peptide Sonic hedgehog (Shh) and are mitotically inactive. Also, we proposed that Shh-expressing basal cells are immediate precursors of other type cells in taste buds, based on their rapid turn-over as compared to Type II and III cells and on the temporal change of cell type marker expression overlapping between cell types during taste bud development. In the present study, to test this hypothesis, we used the tamoxifen-inducible Cre-loxP recombination system to trace the fate of Shh-expressing basal cells in taste buds of adult mice. $ShhCre^{ER}$;R26RLacZ or $ShhCre^{ER}$;CAG-CAT-EGFP mice were treated with tamoxifen to drive Cre-mediated recombination and indelibly label the progenies of Shh-expressing cells by LacZ or EGFP reporter expression. In taste buds, labeled cells were found in fusiform taste bud cells, including Type II and III cells identified by cell type maker expression. Labeled cells were also found in the cells with complex morphology where they appeared to wrap other cells, consistent with Type I cell identity. Thus, we show that Shh-expressing basal cells are immediate precursor cells that differentiate directly into each of three functional cell types in taste buds.

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Symposium 12 "No taste, no smell: When the chemical senses are lost " Sunday 24 June

Quality of life in patients with loss of smell or taste

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A significantproportion of olfactory or taste-impaired individuals have a diminished quality of life (QOL) and ability to perform normal activities of daily living (ADLs). However, a few studies have demonstrated this in some patient populations. We have used patient databasesfrom 2 large smell and taste centers to examine the nature andextent of functional disabilities and alterations in QOL experienced byolfactory-impaired individuals. Themost commonly cited activities impaired by olfactory loss forthe impaired group were detection of spoiled foods (75%), detectionof gas leaks (61%), eating (53%), detection of smoke (50%),cooking (49%), buying fresh food (36%), and using perfume orcolognes (33%). It has been reported that patients with dysosmia ordysgeusia had higher scores on the Beck Depression Inventory,indicating more severe depression, than patients without theseconditions. In addition, patients with chemosensory dysfunctionreported reduced body weight, appetite, and psychological well-being. The otolaryngologist, who isuniquely capable of evaluating smell and taste functions, must be aware of the impact of olfactory dysfunctionon patients' lives.

Poster session II Poster #118

From the olfactory bulb to higher brain centers: a comprehensive axon projection map revealed by genetic single-neuron labeling in zebrafish

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In the olfactory system, odor information is initially represented as a topographic, chemotopic map in the olfactory bulb (OB). In zebrafish, for example, bile acids (putative social cues) and amino acids (potent feeding cues) activate different glomerular clusters in the medial and lateral OB, respectively. However, it remains unclear how the odor map in the OB is transferred to higher olfactory centers. To answer this question, it is essential to analyze in detail the neuroanatomy of individual OB output neurons with special reference to the dendritic innervation of glomeruli and the axonal trajectory to target regions. Here we combine a genetic single-cell labeling method with a state-of-the-art image registration technique to create comprehensive axon projection maps from distinct glomerular clusters to higher brain centers in zebrafish larvae. We find that (1) individual OB output neurons project axons to multiple target regions in the forebrain, including the telencephalon, habenula, and posterior tuberculum (a diencephalic nucleus close to the hypothalamus); (2) OB output neurons innervating the same glomerulus do not necessarily exhibit a stereotyped axon trajectory; (3) OB output neurons innervating distinct glomerular clusters tend to project axons to different, but partly overlapping, sets of forebrain target regions; (4) OB output neurons innervating distinct glomerular clusters exhibit definite terminal fields with extensive overlap in the dorsoposterior zone of the telencephalon, a putative homolog of mammalian piriform cortex. Our results suggest that the chemotopic odor representations in the OB are reorganized, transmitted to multiple brain regions, and processed differently in these regions, presumably according to odor qualities relevant to behavioral outputs.

Poster session II Poster #122

The chemosensory system for flehmen response in the domestic cat

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A domestic cat encounters urine marks deposited by another cats, the cat sniffs the urine marks with considerable interest and then shows an instinctive behavior known as “the flehmen response” in which cats raises the head and holds the mouth partially open with an enrapture expression for a few second. Here we report the chemoreceptor system of the flehmen response in the cat. The flehmen response is also shown by other Felidae animals, Asian elephant and ungulate species, such as buffalo, ram, cattle, and horse. In each of these species, it is well known that males exhibit the flehmen response after sniffing urine of estrous females. In addition to these knowledge, our behavior analysis indicate that male cats do the flehmen response toward not only estrous female urine but also diestrous female and male urine. Numbers of flehmen response are significantly higher in males to estrous urine (7 times/2 min) than males to male and diestrous urine (1 or 2 time/2 min). These results suggest that the flehmen response is dissecting into two types, reproductive and non-reproductive behaviors. We are now purifying flehmen-inducing pheromones from cat urine and examining which olfactory systems, MOS and VNS, detect the pheromones in addition to the studies for biological significances of flehmen response observed in males to same sex urine.

Symposium 13 "Plasticity and modulation in olfactory systems - Linnaeus Symposium" Monday 25 June

Imaging sensory response profiles of adult born and local neurons in the mouse olfactory bulb

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A unique property of the mammalian olfactory bulb (OB) is that it receives continuous supply of interneurons throughout adulthood. Therefore, the hardware of the circuit is dynamically changing on a slow timescale. Little is known about how the OB codes information along time and in particular whether the newborn neurons contribute to this type of processing. In this context, I will present some of our recent efforts to study the sensory physiology of adult born neurons directly as well as those of the existing resident neurons. Using viruses we induced expression of the genetically encoded calcium indicator GCamp3.0 either in newborn neurons or in local populations in the OB. We then combined these viral injections with a chronic window preparation in order to allow direct optical access of the OB for studying physiological responses to odor stimuli. We use in vivo two photon calcium imaging and/or electrophysiology to study newborn neurons at different ages or the existing population over time. I will describe the basal odor responses of neurons imaged repeatedly from distinct local populations of neurons, revealing unique response signatures for different neuronal subtypes in the OB. Repeated imaging of the same local neurons over several weeks already reveals a surprisingly high stability in the response profiles of neurons to a small set of odors and concentration in control mice. I will discuss our current results in the context of long term plasticity of OB circuitry.

Poster session II Poster #178

Mitochondrial Ca^{2+} mobilization is a key element in olfactory signaling

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In olfactory sensory neurons (OSNs), ionized calcium is a key component of a variety of sensory signaling pathways. Odorant receptor - ligand interaction elicits a primary receptor current by opening of cyclic nucleotide-gated (CNG) channels and Ca^{2+} influx into the cilia. This event triggers a number of secondary cellular responses that ultimately shape a neuron's electrical output signal. Therefore, cytosolic Ca^{2+} concentrations are tightly controlled.

Here, we investigate a functional role of mitochondria in shaping the odor-mediated Ca^{2+} response in OSNs. Using genetically engineered mice that express a Ca^{2+}sensitive photoprotein associated to the inner mitochondrial membrane and a dedicated bioluminescence microscope, we establish a novel imaging approach to selectively record Ca^{2+}signals in OSN mitochondria at high temporal resolution. Electrophysiological recordings from single OSNs reveal the functional consequences of mitochondrial perturbation on the odor-mediated primary receptor current and the sensory output. Combined with organelle mobility assays and ultrastructural analysis of individual OSNs, our study identifies mitochondria as key determinants of olfactory signaling.

We show that mitochondria play a vital role in olfactory Ca^{2+}signaling, controlling both primary and secondary Ca^{2+}pathways. When mitochondrial Ca^{2+}sequestration is pharmacologically impaired, the distinct time course of the odor-mediated cytosolic Ca^{2+}signal is significantly changed. Moreover, we report activity-dependent mitochondrial translocation to dendritic compartments upon odor stimulation. Based on electrophysiological recordings, we suggest that Ca^{2+}mobilization by mitochondria exerts a regulatory function that ensures an individual neuron's broad dynamic response range and provides a mechanism of olfactory input-output gain control. Thus, OSNs function as simple stimulus detectors rather than intensity encoders, when mitochondrial function is impaired.

Poster session I Poster #133

Involvement of multidrug resistance transporter activity in the peripheral odorant response in rodents.

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The nasal tissue located at the entry route to the CNS has provided its mucosal epithelia with several xenobiotic metabolizing enzymes. Among others are two members of the multidrug resistance transporter family (MDR), which are toxically relevant efflux transporters, the P-glycoprotein (Pgp) and the multidrug associated protein (MRP). We investigated functional activity of MDR transporters in acute olfactory epithelium slices by means of the fluorimetric calcein–acetoxymethyl ester assay as calcein uptake rates are a measure of MDR activity. In the presence of the Pgp inhibitors verapamil and cyclosporin A, or the MRP inhibitors probenecid and MK 571 calcein uptake measured as an increase in fluorescence intensity in the olfactory epithelium was significantly accelerated . Furthermore, intracellular calcein accumulation in individual olfactory receptor neurons was also significantly increased in the presence of either one of the inhibitors. To test, if MDR transporters may be involved in the olfactory response we examined odorant evoked field potentials using electro-olfactogram recording (EOG) in mice and rat. We monitored EOGs responses from different locations in the main olfactory epithelium in response to two single and a mixture of odorants. In the presence of the MRP inhibitors probenecid or MK 571, peak amplitudes of EOG responses were significantly reduced for all odorants tested, while Pgp inhibitors had only a moderate or no effect. Presence of Pgp or MRP1 encoding genes in the olfactory epithelium was further confirmed by RT-PCR with appropriate pairs of species-specific primers. These result together suggest that MRP-and P-glycoprotein are present and functional in the main olfactory epithelium of rodents and are implicated in the olfactory response. A.M is supported by a grant from the Fondation Edmond Roudnitska (Paris).

Poster session I Poster #55

Electrophysiological screening system to investigate gall midge host plant recognition

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We are investigating gall midge host plant recognition as a basis for development a screening system that allows fast and efficient identification of gall midge host plant attractants. The screening system is based on a blend containing 50 known insect attractants that we are testing on a range of gall midges by the electrophysiological method GC-EAD. So far we have successfully tested this synthetic blend on 10 gall midge species, some of them are closely related with each other some are distantly related species. It is interesting to see how the closely related species that lives on different host plant respond to the compounds compared to the distantly related species that lives on same or similar host plants.

Most of the more than 5000 gall midge (Cecidomyiidae) species are plant feeders and many of them are serious pest. Despite their small size of only 1-2 mm, gall midges show an incredible capability to disperse throughout the world. To protect important agricultural crops and native plants against present and invading gall midges, a reliable monitoring system is off vital important. Males use the conspecific female produced volatiles, pheromones to locate a suitable mating partner. The females use the volatiles emitted by plants to locate a host for oviposition. Based on olfaction, gall midges can differentiate between plants. The differentiation is likely to be based on specific combinations of common plant volatiles that make up unique odor combination for each plant. Gall midge monitoring system based on pheromone have been developed for several species however these systems are aiming at the males, while it is the ovipositing females that select the host plant for the emerging larvae, subsequently causing the damage to the plants. Recent research demonstrates that pest management programs that aim at the female insect are superior to programs aiming at the males as each killed female represents the loss of all her offspring.

Symposium 10 "From odorant receptor to glomerulus" Sunday 24 June

Coding olfaction

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Each olfactory sensory neuron in mouse chooses one of 1,200 odorant receptor genes for expression. Odorant receptor genes are chosen for expression by greatly varying numbers of neurons. The mechanisms that regulate the probability of odorant receptor gene choice remain unclear. We have applied the NanoString platform of fluorescent barcodes and digital readout to measure RNA levels of 577 mouse odorant receptor genes in a single reaction, with probes designed against coding sequences. In an inbred mouse strain with a targeted deletion in the P element (ΔP mice), we find that this element regulates odorant receptor gene choice differentially across its cluster of 24 odorant receptor genes. Importantly, the fold changes of NanoString counts in ΔP or ΔH mice (mice with a deletion in the H element) are in very close agreement with the fold changes of cell counts, determined by in situ hybridization. Thus, the P and H elements regulate the probability of odorant receptor gene choice, not odorant receptor transcript level per neuron.

Poster session II Poster #56

Molecular evolution of sex pheromone receptors in noctuid moths of the genus *Spodoptera*

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Moth sex pheromone communication is recognized as a long-standing model in insect biology and a widespread knowledge has been accumulated on this subject. Notably, it is well known that females of closely related species emit slightly different blends of sex pheromone components, which is necessary to maintain premating isolation. The recent identification of moth pheromone receptors (PRs) opened new routes to understand the molecular bases of this communication system, and the study of PR evolution is of particular interest to highlight mechanisms at the base of reproductive isolation and speciation. In that context, we aim at characterizing candidate PRs in the genus *Spodoptera* and studying their evolutionary history, as a first step toward the exploration of structure-function relationships among moth PRs.

Taking advantage of a large collection of antennal expressed sequence tags, we previously identified four candidate PR genes in *Spodoptera littoralis*. Using a homology cloning strategy, ortholog genes have been identified in the sympatric species *Spodoptera exigua* and isolation of orthologs is in progress in *Spodoptera frugiperda*, a third member of the same genus. Phylogenies of *Spodoptera* and other moth candidate PRs are being constructed using probabilistic methods (Maximum Likelihood and Bayesian Inference) to identify potential deletion/duplication events that could be linked to

the differences in pheromone blend composition of these three species. Functional characterization of *Spodoptera* candidate PRs is in progress using heterologous expression in *Drosophila* olfactory receptor neurons. Finally, we aim at identifying PR regions under positive selection, which may be responsible for the emergence of new ligand receptivity, and finally may contribute to premating isolation and speciation.

Poster session II Poster #364

Influence of the basolateral amygdala on thalamic activity induced by taste familiarity in rats

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Previous lesion studies have shown that basolateral amygdala (BLA) integrity is required for taste neophobia but also for recognising taste familiarity. There are also data supporting the relevance of descendent projections on previous subcortical relay levels for taste learning and memory.

In order to investigate the potential relevance of BLA projections on taste thalamic nuclei processing bilateral excitotoxic BLA lesions by NMDA were combined with Fos-like immunohistochemistry as an index of neural activity. Male lesioned and sham-lesioned Wistar rats received two consecutive exposures to a neophobic 3% cider vinegar solution. The number of Fos-like positive cells in the ventral posteriormedial thalamus, including the parvocellular thalamic taste area (VPMpc) was examined both in lesioned and sham-lesioned brains.

The results showed that drinking a familiar taste solution induced a higher Fos-like immunoreactivity (FLI) in VPMpc than drinking a novel taste solution, while no differences were seen in other thalamic relay nuclei. Moreover, BLA lesions that disrupted recognition of taste familiarity interfered with such familiarity-related FLI increase. Thereafter, the results indicated an involvement of both VPMpc and BLA in a taste recognition memory neural circuit and they support a crucial role of descendent feedback pathways in processing taste familiarity.

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Poster session I Poster #387

Effects of taste stimuli on viscoelasticity of saliva and its ability to inhibit Feline Calicivirus

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Saliva is well-known to be secreted in response to feeding or drinking. However, the effects of taste stimuli on the composition of saliva are not fully understood. This study explored the effects of 5 taste solutions (citric acid, sucrose, sodium chloride, caffeine, and sodium glutamate) on the viscoelasticity, the amount of MUC5B mucin, and the anti-Feline Calicivirus ability of the stimulated saliva. Sweet, sour, and umami taste solutions increased the viscoelasticity of saliva, whereas bitter and salt solutions decreased it. In particular, the sweet stimulus increased the viscosity significantly as compared with the salt stimulus. The flow rates of saliva stimulated by the 5 taste solutions were not related to these changes. We then explored the effects of the 5 taste stimuli on MUC5B mucin in order to reveal the relationship between the viscoelasticity and the amount of MUC5B mucin in the stimulated saliva. The results paralleled those of the viscoelasticity measurment. Sweet, sour, and umami solutions increased the amount of MUC5B mucin, whereas bitter and salt solutions decreased it. This study also explored the effects of the 5 taste stimuli on the ability of saliva to inhibit Feline Calicivirus. Interestingly, sweet, sour, and umami solutions also increased the anti-virus ability of saliva. It is suggested that taste stimuli affect the viscoelasticity and the anti-virus ability of saliva by regulating the secretion of mucin into saliva. In summary, this study reveals that taste stimuli have qualitatively different influences on the composition of saliva.

Poster session I Poster #121

The distribution of protein kinase A regulatory and catalytic subunits varies during development in the mouse olfactory system

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The second messenger cAMP regulates several cellular functions mainly through cAMP-dependent protein kinases (PKA). They are tetramers of two catalytic (C) and two regulatory (R) subunits, of which four isoforms are known: RIalpha, RIbeta, RIIalpha, RIIbeta. PKA distribution inside the brain changes according to cell type, age and physiological status, possibly modifying neuronal responses to variations in cAMP concentration. Therefore, the localization of PKA isoforms was examined during development in the mouse central and peripheral olfactory system, via immunohistochemistry and Western blot. In the peripheral olfactory organs, the olfactory mucosa showed a strong PKA labelling both for R and C subunits. C colocalized with both R subunits. RII was present in the luminal area, while sparse RI aggregates were observed in the inner layers. In the vomeronasal epithelium, only RII and C, but not RI, were detected on the luminal layer. The intensity of labelling increased in the first postnatal week for RII subunits. Like in other brain areas, PKA R subunits were present both in the soluble and in the detergent-insoluble fraction of olfactory bulb. PKA regulatory subunits were variously distributed in the olfactory bulb but never colocalized in the same subcellular structure. In the main olfactory bulb, RIalpha colocalized with C, and showed the main changes during development, being absent at birth, increased in mitral cell layer between postnatally day 5 to 15, and started to decrease at one month of age. A similar time course was detected in the accessory olfactory bulb. In the central olfactory projection areas (bed nucleus of the stria terminalis, olfactory and vomeronasal amygdala and olfactory cortex), RIalpha increased between P15 and P30, declining thereafter. Therefore, the distribution of PKA R and C subunits is modified during development and increases postnatally, in parallel with functional maturation of the olfactory system.

Poster session I Poster #339

Clinical application of a new device for the evaluation of intranasal trigeminal sensitivity

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Introduction: Despite the clinical significance of intranasal trigeminal sensitivity, there is a lack of easy and time-sparing tests. The aim of the present study was to develop a new procedure for the evaluation of intranasal trigeminal sensitivity. It should be tested in healthy subjects and also tried in patients in order to show its clinical usefulness.

Methods: The investigation included seventy-two subjects and patients. Evaluation of intranasal trigeminal sensitivity was performed with a new device measuring the duration of intranasal application of three different concentrations of CO_2 (35%, 45%, 55%; air-flow 1 L/min) twice in each side of the nose. The subjects had to insert a small teflon tube approximately one centimeter in each nostril and breath through the mouth. During pressing a button the flow of CO_2/air-mixture was applied to the subjects' nostril by switching a valve. Simultaneously, the time during pressing the button was recorded by a stop-clock until a stimulus with medium strength was perceived. After an interval of ten minutes the stimuli were repeated in all subjects, resulting in four stimuli of each concentration.

Results: Analysis of variance showed significantly different durations of stimulation for all three concentrations (median duration for the lowest/median/highest concentration: 1.02-1.18/0.69-0.83/0.47-0.53s; $p<0.001$). For all four repeated measurements there was no difference ($p>0.05$). In patients with septal deviation, test results showed longer duration of stimulation ipsilateral to the deviation. Analgesic treatment showed also longer stimulus times in patients after tonsillectomy.

Discussion: The present investigation showed the usefulness of a new device for the clinical assessment of intranasal trigeminal sensitivity. The new device enables the clinician to measure psychophysical responses to trigeminal stimuli in patients and subjects in an easy and quick manner.

Poster session I Poster #57

Oligomerization of the *Drosophila* Orco channel

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Insect Odorant receptors (ORs) form heterodimeric complexes of a ligand binding conventional receptor protein and a highly conserved odorant co-receptor protein (Orco). Odor stimulation of heterologously expressed insect ORs produces a fast ionotropic receptor current accompanied by a slowly developing metabotropic current. Such slow current could also be obtained by cAMP stimulation in cells solely expressing Orco. Our investigation of Orco channel properties showed single events of variable step size indicating that multiple Orco proteins synchronized their activity. We hypothesize that cAMP promotes synchronization by oligomerization. To test the functional role of Orco oligomerization we have engineered a dimer, the simplest oligomeric construct. To grant for a correct orientation of both 7- transmembrane Orco proteins, they were coupled by a 1-transmembrane protein. The Orco dimers were stably expressed in CHO and HEK 293 cells. We performed calcium imaging, immunostaining and patch clamp experiments to characterize the dimer construct. Calcium imaging experiments demonstrate that the Orco dimer construct forms a calcium conducting ion channel. Electrophysiological recordings show a similar channel activity of the dimer construct similar to the activity obtained with Orco monomers.

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Symposium 3 "Chemosensory receptors in non-chemosensory tissues" Saturday 23 June

Mechanisms of post-ingestive glucose sensing

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The detection of ingested sugars is a critical step in the maintenance of glucose homeostasis. Even so, the molecular basis for post-ingestive glucose sensing remains unclear. For example, the sweet taste receptor (T1R2+T1R3) is expressed in endocrine cells of the intestine and pancreas, where it has been implicated in coupling sweetener detection to the secretion of incretin hormones (in the gut) and insulin (in the pancreas). However, other studies indicate that metabolic mechanisms independent of this receptor are sufficient to couple glucose detection to hormone secretion in both tissues. Using $T1R3^{-/-}$ mice, which lack a functional sweet taste receptor, we find that glucose can stimulate secretion of the incretin hormone glucagon-like peptide-1 (GLP-1) from the intestines via two distinct mechanisms. One pathway is restricted to the small intestine, responds to diverse sweeteners and requires the sweet taste receptor subunit T1R3. The second pathway is glucose-specific, sweet taste receptor-independent and upregulated in hindgut with T1R3 deletion. Additionally, we find that pancreatic islets from $T1R3^{-/-}$ mice display dramatically slowed kinetics of glucose-stimulated granule exocytosis, indicating a role for the sweet taste receptor in the modulation of early-phase insulin secretion. Together, our results indicate that the sweet taste receptor is necessary but not sufficient for normal post-ingestive glucose sensing. Support: NIDCD (DC010110), NIDDK (DK077140, DK080899, DK072488), Ajinomoto Amino Acid Research Program.

Poster session II Poster #340

Study on the attenuation of oral fat sensations with Oolong Tea

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Oolong tea is one of the fermented teas made from the leaves of *Camellia sinensis*. This tea is widely drunk in Asia, especially in Japan where it is often consumed unsweetened during meals. This practice is believed to lie in its "refreshing" effects during the course of a meal. It is hypothesized that Oolong tea presents a cleansing effect on oral fat sensations during meals, but so far no studies have established this phenomenon. Here we show through several sensory studies that Oolong tea consumption significantly reduces oral fat sensations compared to water. However, tea drinking with meals is not popular worldwide: many people drink water or carbonated beverages with fatty meals. Presently, we compared the effects of Oolong tea to those of different beverages on oral fat sensation. More specifically, the study focused on the modulation by beverage consumption of perceived oral fattiness elicited by diverse edible oils. Oolong tea and carbonated beverages were used as drinking solutions and water as a control. Subjects exposed their mouth alternately to fat stimuli and drinking solutions, as would occur in a real meal situation. Oral sensations of fattiness, astringency and bitterness were evaluated on a 20 point scale after tasting fat stimuli and drinking the solution. Results are discussed in terms of oral fat sensation attenuation and also cultural differences associated with the perception of Oolong tea.

Poster session II Poster #120

Maintenance of V2R family vomeronasal receptor gene expression in cultured vomeronasal neurons by the interaction with accessory olfactory bulb neurons

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In a previous study using immunoblot and immunocytochemical analyses, we reported that cocultures of the vomeronasal organ (VNO) with accessory olfactory bulb (AOB) neurons resulted in the maturation of vomeronasal sensory neurons (VSNs) and a greater expression of V2R family vomeronasal receptors than cultures with VNO alone. To further characterize the V2R expression, we here investigated the time course of the expression of V2R mRNA in the presence or absence of AOB neurons using RT-PCR analysis. Cultured VNOs and AOB neurons were separately prepared from Wistar rat embryos. After 7 days *in vitro* (DIV) for the VNO and 3 DIV for AOB neurons, coculture was started by transferring some VNOs onto dissociated AOB neurons (day 0 of coculture). Then the cocultured and singly cultured VNOs were maintained for additional 7, 14 and 21 days. The expression of V2R mRNA was already detectable in the VNO before starting coculture. After starting coculture, its expression was maintained not only in the VNO cocultured with AOB neurons for 3 days in coculture but also in the singly cultured VNO for the same number of days. However, the expression of V2R mRNA remarkably declined in the singly cultured VNO during the additional 2 to 4 days in culture, while that in the cocultured VNO showed sustained expression at the corresponding time points, 5 to 7 days in coculture. Moreover, the block of neuronal activities by applying 2 μM tetrodotoxin to the cocultured VNO resulted in a marked decrease in the V2R mRNA expression to a level equal to that in the singly cultured VNO for 14 days in coculture. Previously we hypothesized that the expression of V2Rs in VSNs was induced by interacting with AOB neurons. However, the present results suggest that the receptor expression in VSNs is independent of the interaction with AOB neurons in the early developmental stage, but is maintained by the active interaction with AOB neurons.

Poster session I Poster #195

A functional role for protein synthesis in long-term potentiation at synapses in the mouse accessory olfactory bulb

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Some urinary pheromones of the male block pregnancy of the female in mice. The inability of the mating male to disrupt the pregnancy depends on the memory of his pheromones formed by the female. The pheromonal memory is based on the neural changes in the accessory olfactory bulb (AOB), the first relay in the vomeronasal system. Microcircuits in the AOB include the prominent reciprocal dendrodendritic synapse between mitral cell projection neurons and granule cell interneurons. A previous study has indicated that a protein synthesis inhibitor, anisomycin, infused in the AOB *in vivo* is able to prevent the formation of the pheromonal memory. Other reports have shown that antidromic stimulation of mitral cell axons induces long-term potentiation (LTP) at the mitral-to-granule cell synapse in slice preparations of the AOB. Here we measured field EPSPs derived from granule cells with AOB slices to examine the effects of protein synthesis inhibition on LTP at the mitral-to-granule cell synapse. High frequency stimulation, consisting of a 100 Hz, 100 pulse train applied four times at 3 min intervals, induced LTP lasting for 180 min. Under bath application of anisomycin, high frequency stimulation induced short-term potentiation and early- phase LTP, but significantly failed to induce late-phase LTP. The results suggest that protein synthesis underlies late-phase LTP at the mitral-to-granule cell synapse in the AOB. Further analysis is required to identify newly-synthesized proteins involved in the induction of late-phase LTP. Supported by KAKENHI 23770075.

Poster session II Poster #372

Effect of adenosine monophosphate (AMP) and guanosine monophosphate (GMP) on taste perception of amino acids by mice

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In vitro heterologous expression studies showed that most L-amino acids and D-Ala activate the mouse T1R1+T1R3 receptor when they are mixed with inosine monophosphate (IMP), even though some of these amino acids do not activate T1R1+T1R3 without IMP (Nelson *et al*., 2002). Consistent with this, our previous studies with mice showed that conditioned taste aversion (CTA) to L-Met, L-Val, L-Lys, L-Asp or D-Ala (without purine 5'-ribonucleotides) did not generalize to MSG mixtures [MSG + amiloride (Ami; added to block sodium taste) and MSG + IMP + Ami]. However, CTA to these amino acids mixed with IMP generalized to the MSG mixtures. Like IMP, AMP and GMP also have synergistic effects on umami taste of MSG in humans (Yamaguchi, 1967) and on taste responses of the rat chorda tympani nerve to various amino acids (Yoshii, 1987). The goal of our study was to examine whether addition of AMP or GMP changes taste quality perception of L-Met, L-Val, L-Lys, L-Asp and D-Ala. We have addressed this question using the CTA technique. Separate groups of C57BL/6J mice were exposed to one of the ten conditioned stimuli (five amino acids, 50 mM L-Met, 50 mM L-Val, 50mM L-Lys, 30mM L-Asp and, 50mM D-Ala, mixed either with 2.5mM AMP or with 2.5mM GMP) or to water (control) and injected with LiCl to form CTA. Conditioned mice were presented with the conditioned stimuli, and a mixture of 50 mM MSG and 30 μM Ami with or without 2.5 mM IMP (i.e., MSG+IMP+Ami and MSG+Ami), and their lick responses were recorded. CTA to each of these amino acids, except for L-Asp mixed with AMP or GMP, generalized to MSG+Ami and MSG+IMP+Ami. These results suggest that addition of AMP or GMP changes the taste quality of L-Met, L-Val, L-Lys and D-Ala in a fashion similar to effects of IMP.

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Poster session II Poster #388

Glucose metabolism in T1R3-knockout mice

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Sweet taste receptors are expressed in the gut and pancreatic β-cells and are likely involved in control of glucose absorption and metabolism. In the present study we examined physiological importance of the T1R3 taste receptor in control of blood glucose level. Experiments approved by the ACUC of the Pavlov Institute of Physiology were performed with non-fasted C57BL/6J-*Tas1r3*^{tm1Rfm} mice lacking the entire T1R3 coding region (T1R3-KO; Damak et al, 2003) and inbred C57BL/6ByJ mice (B6; Jackson Laboratory, Bar Harbor, ME) with a wild-type allele of T1R3. In the glucose tolerance test, animals received glucose (1 or 2 g/kg BW) by gastric gavage or by intraperitoneal (i.p.) injection. Blood glucose was measured in samples from the tail 0-120 min post administration.After gavage loading with glucose, T1R3-KO mice tended to have smaller elevation of blood glucose level than B6 mice. In contrast, after i.p. injection blood glucose level was much higher (up to 450% increase of AUC) and declined more slowly in T1R3-KO animals. In the insulin tolerance test, blood glucose was measured 0, 15 and 60 min after 2 U/kg of insulin i.p. B6 and T1R3-KO mice showed similar reduction of blood glucose level in response to insulin. The obtained data show that the T1R3 protein is involved in glucose homeostasis and metabolism *in vivo*. The results also suggest that T1R3 deficiency affect tissue glucose utilization via suppression of glucose-induced pancreatic synthesis of insulin and that T1R3 deficiency may also delay the intestinal transport of glucose. The data support the role of T1R3 in intestinal and pancreatic reception of glucose and the hypothesis that the T1R3 receptor is implicated in fundamental mechanisms of metabolic regulation in response to food intake and thus may participate in the pathogenesis of the most common metabolic disorders, such as obesity and Type 2 diabetes.

Symposium 2 "Coding of taste across mammals: from the tongue to the cortex" Saturday 23 June

Cortical processing of taste stimuli of different qualities produces differential fMRI activation in humans

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We used fMRI to investigate brain activation in response to stimuli representing sweet, sour, bitter and salty taste qualities that ranged from high reward value (sucrose, sweet) to unpleasant, aversive taste (caffeine, bitter). Subjects (hungry or sated) rated the series of taste stimuli using the General Labeled Magnitude Scale while they were delivered in the scanner intra-orally as .3 ml in 1 sec (See Haase et al., *J. Neurosci Meth*, 2007 for stimulus delivery). Functional imaging was conducted on a 3T GE Signa EXCITE short bore scanner using a standard gradient echo EPI pulse sequence to acquire T2*-weighted functional images [(24 axial slices, FOV = 19 cm, matrix size = 64X64, spatial resolution 2.97x2.97x3 mm^3, flip angle = 90°, echo time (TE) = 30 ms, repetition time (TR) = 2 s)]. An event-related paradigm allowed for examination of specific neural events in response to individual stimuli. Whole brain and region of interest (ROI) analyses were conducted using AFNI. Activation was stimulus dependent and observed in primary and secondary gustatory areas previously identified in non-human primates. In addition, activation was localized in regions implicated in reward and learning and memory. ROI analysis demonstrated that activation to stimulus quality (sweet, sour, bitter, salty) was modulated by hunger state and differed by region. Activation was greater overall in the hunger state than in the satiety state and most robust to sucrose in the hunger state. We have identified a number of variables that influence the response in humans, including gender, age, and the psychophysical task of magnitude estimation. Stark contrast between activation in response to taste stimuli of different positive and negative reward value (e.g., sucrose and caffeine) in the hunger state supports differential processing of appetitive and aversive gustatory stimuli.

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Poster session I Poster #373

Cephalic phase blood pressure response to oral sodium stimulus in humans

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High sodium intake is correlated with and identified as a risk factor for hypertension (HTN) and cardiovascular disease (CVD). Human physiology typically responds with anticipatory reflexes to the taste of metabolically important stimuli such as the release of insulin in response to oral glucose exposure. In addition, a urine production reflex is obseverd in rats orally exposed to sodium solutions. We hypothesized that humans will demonstrate a blood pressure (BP) response to oral sodium stimulation in anticipation of an ingested and absorbed salt load and concomitant increase in blood volume. Twelve subjects were tested after an overnight fast without water followed by a 2-minute oral swish with either a 1M NaCl solution or Distilled Water (DW). While maintaining a seated, resting state for 2 hours, BP was manually tested every 10 minutes with a sphygmomanometer on five separate trials of each solution on ten different days. Data indicate that a subset of participants reliably show an initial drop in Mean Arterial Pressure (MAP) within the first 30 minutes of rinsing with NaCl, but not with DW, followed by an increase in MAP. All other subjects indicate an overall lower MAP after rinsing with NaCl compared to DI. This lower MAP after rinsing with NaCl is consistent with the relaxation of vessel walls to accommodate the anticipated increase in blood volume. This is subsequently followed by a corrective increase in MAP when the salt bolus, which was tasted and not swallowed, does not appear in blood. These data suggest that there are anticipatory autonomic reflexes in the cardiovascular system to the tastes of salt and water. Future testing will explore other salty stimuli such as 1M KCl to address the specificity of cardiovascular reflexes to the taste of solutions.

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Poster session II Poster #436

Appetitive long-term flavour conditioning modulates human visual evoked potentials

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Background Learned associations betweenimages and the flavour of food items are an integral part of human life. The association invites questions concerning the effect that flavour conditioning may have on brain processing of visual information. Such effects have barely been addressed experimentally and particularly there is a lack of knowledge about the long-term effects of flavour conditioning on human visually evoked brain potentials. In order to test whether flavour conditioning may change image processing, EEG-recordings were performed over visual cortex areas in young healthy subjects.

Experiment EEG-responses evoked by images were recorded from 11 young subjects (19-27 years, mean 24.5 years, sem 0.8 years, 5 males) by means of a 64 channel system (ANT, Holland) before and after conditioning induced by paired stimulations with an image (conditioned stimulus) followed by ingestion of an unfamiliar food item (unconditioned stimulus).

Results The results showed that amplitudes of visual evoked potentials (the P3-component) were significantly enhanced by conditioning. Furthermore, the time from image onset to the start of P3 was significantly shortened and the duration from start to peak was prolonged. Mean power recorded during image presentation rose significantly in the delta and theta frequency ranges. Low resolution electromagnetic tomography revealed that brain sources responsible for generation of P3 before and after conditioning did not relocate in visual cortex as a result of the learning process. However, conditioning enhanced the current density of the dipoles.

Conclusion We have found that appetitive long-term flavour conditioning modulates human visual evoked potentials and it is suggested that the appetitive flavour conditioning may have potentiated the synaptic activity in the involved visual cortex networks and that this led to an increased speed of image processing.

Poster session I Poster #177

Ric-8B is essential for mouse embryogenesis

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Odorant receptors (ORs) belong to a large family of G protein coupled receptors. Odorant signal transduction initiates when odorants bind to ORs leading to the activation of a specific heterotrimeric G protein, Golf. The action of Gαolf subunit is positively regulated by a guanine nucleotide exchange factor (GEF) Ric-8B, which is specifically expressed in mature olfactory sensory neurons. In adult mice, Ric-8B and Gαolf are also coexpressed in regions of the brain such as the striatum, nucleus accumbens and olfactory tubercle. To study the role of Ric-8B in vivo we generated transgenic mice using the Gene Trap technology for Ric-8B gene inactivation. The knockout resulted in a mouse strain that is not viable, while heterozygote mice reproduce normally and show no apparent olfactory deficiencies. Since Ric-8B is crucial for embryo development, we investigated the role of Ric-8B during embryogenesis. We found that knockout embryos die around embryonic day 8.5. At this stage, Ric-8B expression is restricted to certain regions of the nervous system, such as the ventral region of the neural tube, the midbrain and hindbrain.

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Poster session II Poster #404

Ethylmaltol enhance salivary hemodynamic responses in combination with sucrose detected by near-infrared spectroscopy

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The perceived intensity of a taste is enhanced only if the aroma is perceptually similar to the taste. The sweet scent, for example, caramel-like aroma enhances the perceived intensity of sweetness. The manner of integrating gustatory and olfactory signals of foods in the brain has been suggested to depend on one's previous experiences with taste and aroma pairings. To elucidate the effects of ethylmaltol, caramel-like aroma, on sweet taste caused by the central integration of flavor, we performed optical imaging of salivary hemodynamic responses using near-infrared spectroscopy (NIRS) in seven panelists. The recording with NIRS of salivary hemodynamic signals, which have been reported to be accompanied by saliva secretion from the parotid gland in response to taste stimuli, enabled us to test panelists in a normal, seated position with minimal restriction of movement during drinking. First, we observed concentration-dependent increases in the amplitude of the responses to the sucrose solutions from 0 to 8% in the seven panelists. Second, when ethylmaltol was added to the 4% sucrose solution, we observed a statistically significant increase in the amplitude of the responses as compared to that to the odorless sucrose solution in the seven. The addition of ethylmaltol to tasteless solution caused no significant change in the amplitude of the salivary hemodynamic responses. These results indicate that sweet aroma of ethylmaltol enhances the salivary hemodynamic responses especially in combination with sweet tastants, in a similar way as the increase in the concentration of the tastants enhances the salivary hemodynamic responses. Thus the optical imaging of salivary hemodynamic responses provides a sensitive means to detect the effect of added aroma on the taste-related salivary responses, dependent on the central integration of the combination of aromas and tastes.

Poster session I Poster #437

Chemical stimulation of the pharynx to trigger the swallowing reflex and its inter-individual variation in humans

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Both peripheral and central inputs can activate the swallowing central pattern generator (CPG) in the brain stem and the CPG can trigger swallowing. We have reported that swallowing intervals (SIs) in repetitive voluntary swallowing at maximum frequency were prolonged with diminution of peripheral inputs, and varied greatly among subjects. It can be assumed that not only cortical drive to the CPG but also triggering level of CPG neurons to initiate swallowing varies greatly in subjects. The aim of the present study was to investigate the possible neuronal mechanisms that cause the inter-individual variation. SIs were measured by submental EMG recordings during swallowing. Chemical stimulation of the laryngopharynx (LP) consisted of infusion of distilled water (DW) and 0.3 M NaCl solution into the LP through a fine tube. DW stimulates water receptors and a 0.3 M NaCl solution inhibits the activity of water receptors. In voluntary swallowing test, 0.3 M NaCl was infused at a very slow rate (0.2 ml/min) that scarcely caused mechanical effect of infusion. In this case, peripheral inputs would not be involved in initiation of voluntary swallowing. Infusion of 0.3 M NaCl at 1 ml/min, that caused moderate mechanical stimulation, reflexively initiated swallowing and gave rise to great inter-individual variation in SI. There was a linear correlation between SIs in the voluntary swallowing and in the reflexive swallowing. Therefore, neither peripheral inputs nor cortical drive is responsible for the inter-individual variation in initiation of swallowing. It appears that triggering level of CPG neurons to initiate swallowing varies greatly among subjects. Infusion of DW into the LP shortened SI. Facilitation of swallowing by DW stimulation appeared strongly in subjects showing difficulty (longer SI with infusion of 0.3 M NaCl). Therefore, inter-individual variation becomes moderate during DW stimulation in voluntary and reflex swallows.

Poster session II Poster #248

Do good pictures make odors less good? Investigation of hedonic contrast on odors

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When we contact with the odor, we often evaluate the odor in relative comparison with other various stimuli in the environment. The relative comparison induces the framework of assessment. Therefore, the rating ofodors is influenced by the context. In one of this phenomenon, hedonic rating for the target stimulus is less good when it continuously followed better stimuli than when it appeared alone. Through the contact with the prior stimulus, the framework for the evaluation of the following stimuli is changed. This effect, called “hedonic contrast”, occurs as far as participants regard the target and context stimuli are in the same category from the mono-sensory modality. It is unclear that whether hedonic contrast occurs as long as target and context stimuli related conceptually regardless of sensory difference. This study investigated the hedonic contrast during odor evaluations when context stimuli were from mono- or cross-sensory modality. In the latter condition, participants rated the hedonics for the plants-like neutral odors following the flowers pictures (Same-category group) or animal babies pictures (Different-category group). As a result, the hedonics of odors in the same-category group did not differ from them in the different-category group. It is suggested that the odor evaluations might not be affected by the hedonics of the context stimuli from different sensory modality. It seemed to be difficult to recognize that the odors used in the experiment belong to the same category as flower pictures. If contrast is not shown even if the odors relate to pictures more clearly, there is possibility that the occurrence of contrast depends on sensory factor rather than conceptual factor.

Poster session I Poster #405

Basal cell markers and Sox2 during taste bud development and maturation

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In mammals, taste bud cells are maintained by continuous cell renewal, and elongated cells are differentiated from the basal cells within taste buds. Characterization of the basal cell is important for understanding the mechanism of taste bud maintenance. We previously reported that Shh and Prox1 are expressed in the basal cells of taste buds in adult mice, and that these basal cell markers appear at E12.5 and E14.5 during development of the fungiform papillae (FF) and soft palate (SP), respectively. However, timing of the basal cell marker appearance during development of the circumvallate papillae (CV) is not certain. Recently, Sox2 was reported to be required for development of taste bud cells. The relationship between the basal cell markers and Sox2 is also not certain. In the present experiment, we examined the expression of Prox1, Shh and Sox2 during development of the CV, FF and SP. Prox1 expressing cells were Sox2 positive at the beginning of Prox1 expression in taste bud primordia in the FF and SP, while Sox2 expression was not restricted to taste bud primordia in the SP. Shh and Sox2 were co-expressed in the entire epithelium of CV forming region at E12.5. Prox1 expressing cells were interspersed in this region. At E14.5, Prox1 was expressed only in the apical epithelium of CV forming region. At E17.5, scattered expression of Prox1 was observed in the trench wall where Shh and Sox2 were expressed broadly. Thus, we show that: 1) the beginning of taste bud development may be indicated by Prox1 expression before the expression of Shh and/or Sox2 is focused to taste bud primordia, 2) during the CV development, the onset of the basal cell marker expression in the apical epithelium precedes that in the trench wall.

Poster session II Poster #180

The accessory olfactory system in the Japanese toad (*Bufo japonicus*) consists of two types of sensory epithelia

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The recessus olfactorius (RO) is a small pouch observed in the antero-lateral olfactory epithelium of anurans (Helling,1938). Following studies have shown that the RO is lined with ciliated epithelial cells. Location of the RO in the nasal cavity and the presence of ciliated cells both suggest sensory function of the RO. However, whether the RO is a part of the main olfactory system has not yet been studied. In Japanese toads we studied central projection from the epithelium lining RO (ROE) by using fluorescent carbocyanine dye, and found that the neuronal fibers from the ROE did not project to the main olfactory bulb (MOB) but to the accessory olfactory bulb (AOB). The projections from the ROE were found in the anterior region of the AOB and also in the anterior half of the posterior region. In electron microscopy we compared the tissue collected from the ROE with those from the olfactory epithelium (OE) and the vomeronasal epithelium (VNE). The ROE and the VNE contained both ciliated cells and microvillous cells, while the OE contained ciliated cells as seen in the mammalian OE. Some microvillous cells in the ROE were enveloped by supporting cells. Those microvillous cells were reminiscent of crypt cells in the fish OE. The expression of α-subunit of G-proteins ($G_{\alpha olf}$ and $G_{\alpha o}$) in the ROE was examined by immunohistochemistry and *in situ* hybridization: $G_{\alpha olf}$ was expressed in the apical cell layer of the ROE and $G_{\alpha o}$ in the basal cell layer. Such a differential expression pattern of $G_{\alpha olf}$ and $G_{\alpha o}$ has been also shown in the VNE of Japanese toads by our previous study. These results suggest that the ROE of Japanese toads is not a part of the main olfactory system, but of the accessory olfactory system.

Poster session II Poster #194

Vasopressin serves to promote the induction of synaptic plasticity in the mouse accessory olfactory bulb

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Vasopressin secreted from the posterior pituitary gland into the systemic circulation regulates peripheral targets, notably the kidney and the blood vessels. However, this nonapeptide also has important behavioral functions that are not liked to its classical neurohypophyseal release.In fact, a substantial body of evidence has accumulated showing that central vasopressin facilitates social recognition and modulates a variety of complex social behaviors in mammals. Previous studies have reported the presence of vasopressin receptors and neurons in the accessory olfactory bulb (AOB). However, their functions are largely unkown. The AOB,the first relay in the vomeronasal system, has been demonstrated to be a critical site for pheromonal learning in female mice. Pheromonal learning is based on the synaptic plasticity of glutamatergic transmission from mitral to granule calls in the AOB. These findings prompted us to examine the effect of vasopressin on synaptic plasticity of glutamatergic transmission from mitral to granule cells in the AOB. The strength of synaptic transmission from mitral to granule cells can be analyzed by lateral olfactory tract (LOT)-evoked field potentials in parasagittal AOB slices. We measured the maximal initial slope of field EPSPs of granule cells to monitor the strength of glutamatergic transmission from mitral to granule cells. Sub-threshold LOT stimulation, consisting of a 100 Hz, 100- pulse train applied twice at a 3-min interval, induced only short-term potentiation that decayed back to its control value. In contrast, the pairing of the sub-thershold LOT stimulation and bath application of vasopressin produced robust long- term potentiation (LTP) that lasting for at least 3 hours. These results suggest that vasopressin facilitates the induction of LTP at the mitral to granule cell synapse in the AOB.

Contributed talks V "Human olfaction" Monday 25 June

Encoding the odor of musk

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Despite the availability of a large set of data there still remains no consensus on molecular parameters required for the musky odor quality.[i] Main challenges for this task represent non-deorphanized olfactory G-protein coupled receptors, as well as the broad structural diversity of musk ligands. Macrocyclic aliphatic compounds, polycyclic benzene derivatives, nitro arenes and the recently identified alicyclic musks represent the major structural groups of this large family. Even though one might assume different binding features for structurally distinct musks, the typical musky odor they invoke suggests that there is a unique set of receptors, which if activated encodes the recognizable scent. Using molecular modeling and pharmacophore search we were able to find a common set of musk-specific features that covers a structural variety across the musk subfamilies.

We used pharmacophore features as input variables together with other carefully selected molecular descriptors[ii] and were able to build a predictive model on a data set including all but one musk subfamily. Applying the model on a new diverse data set we managed to classify the exempt subfamily of musks as such.

The results of this structure-activity relationship study reveal minimal structural requirements for a molecule to display a musky odor. We show that the complexity of such problem requires a strong chemical intuition and the use of various computational tools. Based on our conclusions we aim to design a new subfamily of sustainable musk molecules where we eliminate existing biodegradability issues, while at the same time preserve the valuable perfume performance assets such as odor long-lastingness and tenacity.

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Poster session I Poster #341

NaCl taste thresholds in mice are linked to chromosomes 1 and 9

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Molecular mechanisms of salty taste in mammals are not completely understood. Although recent studies have highlighted the role of the epithelial sodium channel (ENaC) in the peripheral salt taste pathway (Bosak et al, 2010; Chandrashekar et al, 2010), these studies also demonstrated that ENaC alone cannot account for the entire taste response to NaCl. We are utilizing a genetic approach to study these salty taste mechanisms. We developed a high-throughput procedure to measure NaCl taste thresholds (Ishiwatari and Bachmanov, 2009) and used it to measure NaCl taste thresholds of mice from 13 inbred stains (Ishiwatari and Bachmanov, 2012). We found that mice from the 129P3/J (129) strain have higher NaCl thresholds (30 mM) than C57BL/6ByJ (B6) mice (7 mM). We next produced F1 hybrids between the 129 and B6 strains, and then backcrossed the F1 hybrids onto 129 or B6 strains to obtain two reciprocal backcross (N2) generations, F1x129 and F1xB6. The N2 mice were phenotyped and genotyped, with genetic markers across all chromosomes, to conduct a full genome scan. Significant linkages were found in both F1x129 and F1xB6 mice, to chromosomes 1 and 9 respectively. To isolate these QTLs, we began construction of 129.B6-Chr1 and B6.129-Chr9 consomic strains using serial backcrossing. This involved production of additional backcross generations (N3 - N5), which were phenotyped and genotyped for markers on the chromosomes 1 (backcross to 129) or 9 (backcross to B6). This data, combined across generations, confirmed linkage to both chromosomes. Neither of these chromosomes contain ENaC genes; therefore our research points to genetic variability related to the salt taste phenotype beyond ENaC, and will facilitate novel candidate gene discovery.

Contributed talks IV "Olfactory receptors, ligand interactions and transduction mechanisms" Monday 25 June

Scaffolding by MUPP1 regulates assembly of odorant receptor-associated protein complexes and signaling in olfactory neurons

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The olfactory signal transduction cascade transforms odor information into electrical signals by a cAMP-based amplification mechanism. The mechanisms underlying the very precise temporal and spatial organization of the relevant signaling components remains poorly understood. Here, we report that individual PDZ domains of MUPP1 interact with a broad variety of murine olfactory receptors. Our co-immunoprecipitation experiments identified a macromolecular assembly of signal transduction components in olfactory neurons, organized via MUPP1. Disruption of the PDZ signaling complex through an inhibitory peptide strongly impaired odor responses and changed the activation kinetics of olfactory sensory neurons. In addition, our experiments demonstrated that response termination is dependent on PDZ-based scaffolding. These findings provide new insights into the functional organization and regulation of olfactory signal transduction.

Symposium 18 "Olfactory neuroethology" Tuesday 26 June

A new frontier for chemical ecology in birds: from molecules to behavior

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Personal odors can be influenced by genes of the major histocompatibility complex (MHC), a cluster of genes essential for immune function. These odors impact recognition and preference in mammals, lizards and fish, but whether MHC-associated odors could play a similar role in birds is currently not known. The tube-nosed seabirds (Order: Procellariiformes) are an ideal group for investigating this possibility. These birds mate for life, have a remarkable sense of smell, and some species can tell each other apart by smell. This latest finding suggests that personal scent may play a role in social behaviors, which, until recently, has not been seriously considered in birds. This presentation will emphasize recent investigations into the genetic and chemical basis for individual odor recognition in a common burrow- nesting species, the Leach's storm-petrel *(Oceanodroma leucorhoa)*. We characterized partial genomic fragments from two MHC class IIB gene duplicates in this species. Locus-specific primers allowed us to assign10 alleles from the polymorphic second exon (peptide-binding region, or PBR) to the Ocle-DAB1 MHC gene and 15 alleles to the Ocle- DAB2 MHC gene. Both loci displayed characteristics typical of functionally important MHC genes and up to 23 out of 90 codons of the PBR showed decisive evidence for positive selection. A robust sample size of 300 mated pairs is now being genotyped to establish whether MHC is involved in life-long partner choice. Preliminary results suggest evidence for directional selection of particular genotypes, rather than a strict preference for heterozygosity. In addition we are using forensic methods to analytically profile individual scent by genotype.

Poster session II Poster #374

Leptin is essential in maintaining basal sweet sensitivity in mice.

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Leptin (Lep) is an anorexigenic mediator that reduces food intake by acting on hypothalamic receptor, Ob-Rb. Lep is shown to selectively suppress sweet taste responses in lean mice but not in Lep receptor-deficient *db/db* mice. In marked contrast, endocannabinoids (EDs) are orexigenic mediators that act via CB_1 receptors in hypothalamus and limbic forebrain. In the peripheral taste system, EDs also oppose the action of Lep and enhance sweet taste sensitivities in lean mice but not in mice genetically lacking CB_1 receptors. However it has not fully been made clear that expression of related molecules in taste cells and action of endogenous Lep and EDs on taste nerve responses. So to address these issues, we first performed immunohistochemistry. The results showed that about 40 % of taste cells expressing Ob-Rb coexpressed T1r3 and a subset of taste cells expressed biosynthesizing enzyme (DAGL) and degrading enzyme (MAGL) of ED (2-AG). We next examined effect of antagonists for Ob-Rb (leptin L39A/D40A/F41A) and CB_1 (AM251) on the chorda tympani (CT) responses. The results demonstrated that lean mice showed significant increases in CT responses to sweet compounds of Ob-Rb antagonist, while they showed no significant changes in CT responses after CB_1 antagonist. In contrast, *db/db* mice showed clear suppression of CT responses to sweet compounds after CB_1 antagonist, and observed enhanced expression of DAGL in taste cells. These findings suggest a possibility that circulating Lep may act as a modulator in wild-type mice that tonically affects basal sweet sensitivity, while EDs whose production may be potentiated under defects in the Lep system as observed in *db/db* mice.

Symposium 16 "Taste and beyond - integration of nutrient sensor functions in oral cavity and gut - Ajinomoto Symposium" Tuesday 26 June

Modulation of sensitivities of oral and gut taste sensors by endogenous factors

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The taste organ is a peripheral target for leptin (Lep), an anorexigenic mediator, and endocannabinoids (EDs), orexigenic mediators. Lep selectively suppresses behavioral, taste nerve and taste cell responses to sweet compounds in wild-type mice but not in *db/db* mice with defects in the Lep receptor, Ob-Rb. Opposing the action of Lep, EDs enhance sweet taste sensitivities in wild-type mice but not in mice genetically lacking CB1 receptors. Administration of an antagonist for Ob-Rb increased responses to sweet compounds in lean mice, but not in *db/db* mice, while that for CB1 receptor do not affect taste responses in lean mice but reduced sweet taste responses of *db/db* mice. These findings suggest that circulating Lep may act as a dominant taste modulator that affects basal sweet sensitivity in lean mice, while EDs may not normally affect taste responses in lean mice, but become effective only when activity of sweet inhibitory system decreases by some reasons, as shown by *db/db* mice. Recently, gut enteroendocrine cells are shown to express sweet taste receptors and Ob-Rb and respond to sweet compounds followed by increases of hormone release and glucose absorption. We found that Lep acts on STC-1 enteroendocrine cells and selectively suppressed Ca^{2+} responses of the cells to sweeteners without affecting responses to bitter stimulus. These findings suggest a possibility that Lep may also influence sensing and absorption of nutrients in the gut through the gut taste system. Collectively, Lep modulation on peripheral sweet taste reception through the oral and gut taste systems may play an important role in regulating energy homeostasis.

Symposium 19 "Preference for umami taste controlled by chemical senses - Ajinomoto Symposium" Tuesday 26 June

Multiple effects of umami compounds in the forebrain

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Recent behavioral studies suggest that taste substances affect the animal's instinct and motivated behaviors by stimulating oral taste receptors and chemoreceptors in the gastrointestinal (GI) tract. The forebrain (orbital cortex, amygdala, hypothalamus, hippocampus, etc.) involved in these behaviors receives taste information from the brainstem taste centers, and also visceral information from the vagus nerve. In the present study using rats, neuronal activity was recorded from these forebrain taste centers and parabrachial gustatory nucleus during ingestion of taste solutions including umami tastes. In the orbital cortex and amygdala, patterns of neuronal responses to various taste solutions suggest that taste is encoded based on palatability of the taste chemicals, while the parabrachial nucleus is involved in taste quality discrimination. In the hippocampus, taste quality might be encoded based on hedonic value, which is similar to the orbital cortex and amygdala. However, the activity of most hippocampal taste neurons was location-specific.

To investigate how chemosensory information of taste substances from the gut is encoded and processed in the forebrain, we recorded neuronal activity in the rat central nucleus of the amygdala (CeA) and lateral hypothalamic area (LHA) during intragastric administration of various nutrients. After 12-hr fasting, rats received intragastric infusion of various solutions including monosodium glutamate (MSG, 60 mM), NaCl (60 mM), amino acids, or saline up to 1 % of the body weight for 10 min. Neuronal activity was recorded for 60 min from 10 min before intragastric infusion to 50 min after the start of intragastric infusion. A total of 26 and 11 neurons were recorded from the CeA and LHA, respectively. Of these, activity of 20 (77 %) of the CeA and 10 (91%) of the LHA neurons changed significantly during the post-administration period compared with the pre-administration period. Furthermore, in the CeA, the mean response latency to the amino acid solutions was significantly shorter than that of the other solutions (amino acids, 9.9±8.2 min; others, 17.0±6.8 min) and the mean response duration to the amino acid solutions tended to be longer than that of the other solutions (amino acids, 21.3±11.0 min; others, 9.5±4.3 min). The results indicated that many neurons in these regions change their activity in response to intragastric administration of nutrients, and suggest that CeA neurons are more sensitive to amino acids than the NaCl solution and saline. Furthermore, amygdalar and hypothalamic neurons responded to intragastric infusion of umami solutions. These results suggest that chemical information outside and inside the body affects instinct and motivated behaviors through the forebrain.

Poster session II Poster #406

Characterization of clonal cell lines derived from murine taste buds with imaging system of intracellular calcium

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We have recently established a number of clonal cell lines from taste buds of a young *p53*-deficient mouse (TBD cell lines). In the presentstudy, we physiologically analyzed TBD cell lines. The expression patterns of some taste receptor molecules and signal transduction-related molecules were examined in two TBD cell lines, TBD-a5 and TBD-c1 with RT-PCR. Both TBD-a5 and TBD-c1 cell lines expressed some taste receptors and the candidates for five basic tastes, such as T1R family, T2R family, HCN4 and ENaC. Hence, to examine whether TBD cell lines can response to taste stimuli, we performed calcium-imaging analysis. Sweet, bitter, umami stimuli did not elicit elevation of intracellular Ca^{2+} in either TBD-a5 or TBD-c1 cell lines. On the other hand, TBD-a5 and TBD-c1 cell lines responded to sour taste stimuli, and a transient elevation of intracellular Ca^{2+} was elicited. The results suggest that these TBD cell lines can respond to sour taste stimuli, and they are useful *in vitro* models of taste receptor cells.

Contributed talks III "Mixed session" Monday 25 June

Bitter compounds and their interactions with bitter taste receptors

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Bitter taste is one of the basic taste modalities, important for avoidance of poisonous foods. Bitter-taste perception is mediated by a number of GPCRs of the hTAS2R gene family, which varies from 3 to 50 receptors, depending on the species. How can few receptors detect numerous structurally diverse bitter compounds? Why are some of the receptors broadly-tuned, while others are capable of binding only a small number of ligands? What are the structural and molecular details responsible for the complicated overlaps in ligand-receptor associations? Molecular modeling and computational docking of bitter ligands into associated receptors, are used to elucidate the molecular details of recognition. In parallel, BitterDB http://bitterdb.agri.huji.ac.il/bitterdb/, the publicly available resource of bitter compounds, is used to analyze the structures and chemical properties of bitter compounds in an effort to characterize bitterness. These approaches shed light on the molecular details of bitter taste recognition and provide new directions in the identification and design of agonists and antagonists for bitter taste receptors. Levit, A., Barak, D., Behrens, M., Meyerhof, W., and Niv, M.Y. (2011). Homology model-assisted elucidation of binding sites in GPCRs. Methods in Molecular Biology, in press. Wiener, A., Shudler, M., Levit, A., and Niv, M.Y. (accepted in NAR). BitterDB, a database of bitter compounds.

Poster session II Poster #58

Modulation of pheromone responses in antennal trichoid sensilla of the hawkmoth *Manduca sexta* with two prospective Orco antagonists MIA / HMA and the prospective Orco agonist VUAA1.

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Manduca sexta females use a species-specific blend of pheromones to attract conspecific males. For pheromone detection male antennae have very sensitive trichoid sensillae which are innervated by two olfactory receptor neurons (ORNs). One of each ORN is sensitive to the main pheromone component bombykal (BAL). The BAL activates an olfactory receptor-coreceptor complex (OR/Orco) in the outer dendrites of the ORNs. However, the BAL-dependent transduction cascade is still under debate. We investigated the role of Orco in pheromone transduction with application of the prospective Orco antagonists MIA and HMA and the Orco agonist VUAA1 during BAL stimulation. The extracellular single sensillum recordings were performed for two hours at Zeitgebertime (ZT) 1-3 (end of activity phase) and ZT 9-11 (resting phase) with a non-adapting BAL stimulus protocol. At both ZTs the prospective Orco blockers MIA and HMA (10 µM each) decreased both the BAL-dependent sensillum potential amplitude (SPA) and the BAL-dependent action potential frequency (APF of first 5 APs). The background activity (APF between two stimuli) was also decreased for all recordings performed with MIA and HMA. Application of VUAA1 leads to a transient increase of the SPA at both ZTs. Additionally, the APF at ZT 1-3 transiently increased VUAA1- dependently, whereas in recordings at ZT 9-11 it was significantly decreased. The background activity was significant increased by VUAA1 at ZT 1-3, however there was no effect on the background activity at ZT 9-11. To challenge our findings, future hawkmoth BAL receptor and Orco heterologous expression assays will further examine whether MIA and HMA are indeed specific Orco antagonists and VUAA1 is a specific Orco agonist. [Support: DFG grants STE 531/20-1 to MS and Schwerpunktprogramm SPP 1392]

Poster session II Poster #216

Brain activation (fMRI) from chemosensory stimulation in idiopathic environmental intolerance attributed to chemicals

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Idiopathic environmental intolerance (IEI) attributed to chemicals with odorous/irritating properties is a medically unexplained condition. IEI is prevalent predominantly among women, and may result in seriously compromised quality of life due to, often severe, health symptoms from low-level exposure to non-toxic common substances, such as perfume and cleaning agents. No study to date has used functional magnetic resonance imaging (fMRI) to study brain activation in IEI from chemosensory stimulation. The objective of the present study was to investigate the pattern of brain activation in IEI from non-toxic chemosensory stimulation. Brain activation was studied with fMRI during exposure to olfactory (amyl acetate) and chemosomatosensory (CO2) stimuli in 26 female participants who met the IEI criteria, and 30 age-matched healthy female controls. None of the participants were anosmic. Stimuli were presented with a dynamic olfactometer (OM2s, Burghart Instruments) in a block design. The IEI group showed significantly lower BOLD signal than controls in the dorsolateral prefrontal cortex when exposed to amyl acetate (uncorrected for multiple comparisons; threshold of p

Poster session I Poster #375

Targeted taste cell specific over-expression of BDNF in adult taste buds affects taste bud size and promotes gustatory innervation

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Brain-derived neurotrophic factor (BDNF) is the most potent neurotrophic factor in the peripheral taste system during embryonic development. It is also expressed in adult taste buds. While developmental expression of BDNF has been extensively studied, there is a lack of understanding of its role in the adult taste system. To study the roles of BDNF in the adult taste system, we generated transgenic mice with taste cell specific over-expression of BDNF. Transgene expression was driven by an a-gustducin promoter coupling mature BDNF expression to the postnatal expression of gustducin (Gust-BDNF). We show that different transgenic lines overexpress different levels of BDNF transcripts in their taste buds. We also show that taste buds in high BDNF expressing transgenic mouse lines are significantly larger and have a larger number of taste cells as compared to those of wild type controls. To examine whether innervation was affected in Gust-BDNF mice, we used antibodies to neural cell adhesion molecule (NCAM) and ATP receptor P2X3. The total density of general innervation and specifically gustatory innervation were markedly increased in high BDNF expressing transgenic mice compared to low BDNF expressing transgenic mice and wild type controls. TrkB and NCAM gene expression in laser capture microdissected taste epithelia were significantly upregulated in high BDNF over-expressing mice compared to controls, indicating a direct increase in response to BDNF overexpression. We propose that Gust-BDNF transgenic mouse models can be employed to dissect the specific roles of BDNF and its downstream signaling pathways in the adult taste system.

Poster session II Poster #288

Finding a rose in the woods—visual-olfactory integration of positive emotion

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Crossmodal integration is a process ubiquitous in animals with multiple sensory systems, facilitating perception especially when it is challenged with limited stimulus input. However, there is little research in multisensory integration of emotion. Applying functional magnetic resonance imaging (fMRI) techniques, this study examined visual and olfactory crossmodal integration of positive affective versus neutral stimuli, contrasting perception of two pleasant flower odors and two neutral wood odors (at perithreshold concentrations for identification) . Participants (N=29) performed an odor categorization task in an fMRI scanner: they smelled an odor from one of the two categories while viewing a picture that is congruent or incongruent to the odor, followed by a category decision (floral or wood). Reaction time was faster for floral versus wood odors (P<.001), and for congruent versus incongruent stimuli (P<.05). Similarly, accuracy was enhanced for floral than wood odors and congruent than incongruent stimuli (P's<.005). SCR data showed an interaction effect (category X congruency): greater congruency effect for floral than wood odors, indicating stronger autonomic arousal associated with congruency in positive affect (P<.01). fMRI analysis demonstrated enhanced right orbitofrontal cortex (OFC) activation for congruent versus incongruent stimuli. Additionally, greater activation in the left amygdala appeared for floral (vs. wood) odors, when the odors were accompanied by congruent (vs. incongruent) pictures. These findings suggest that congruent visual and olfactory stimulation preferentially enhances response to pleasant than neutral smells, representing crossmodal integration of positive emotion. In particular, the amygdala is involved in facilitating emotion encoding by binding bimodal sensory inputs, and the OFC aids in integrative sensory processing, enhancing olfactory perception of the stimuli and driving increased response accuracy.

Poster session I Poster #303

The relation of sexual orientation, gender nonconformity and olfactory abilities

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A vast body of research has shown that women tend to outperform men in various olfactory abilities. Further, homosexuals often exhibit gender atypical traits, as might well be the case with olfaction. We hypothesised that both in men and women sexual orientation would be correlated with olfactory scores and odour awareness but it would go in the opposite direction in women. The Sniffin' Sticks test was used to assess the olfactory threshold, discrimination and identification (TDI) of 40 homosexuals (F=20) and 40 heterosexuals (F=20) aged 20-35. Further, self-report Gender Nonconformity Scale (GN) and Odour Awareness Scale (OAS) were administered. A GLM analysis was performed, which yielded a significant sex difference and sex*sexual orientation interaction. Post-hoc tests revealed that this was due to a difference in the olfactory threshold, with heterosexual men being less sensitive than heterosexual women (p= .017); in the TDI, with heterosexual men scoring less than both homosexual men (p= .009) and heterosexual women (p= .016) and, finally, in the OAS score, with heterosexual men scoring less than heterosexual women (p= .038). Furthermore, it was found that the self-reported sexual orientation correlated with the identification and TDI scores in men, as did the GN score, with homosexuals and those tending towards nonconformity outperforming the conformist ones. In women, the GN score correlated with the threshold and TDI scores, with the conformist ones outperforming those tending towards nonconformity. The results suggest differences between male and female homosexuality and that olfactory abilities correlate with gender nonconformity in both sexes.

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Poster session I Poster #217

Modular encoding of the odor signatures by major urinary proteins (MUPs) in mice may create an individual bar-code: from fundamental aspects of olfaction to innovative prospects in biomedicine

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Major urinary proteins (MUPs) of the mouse represent a large group of the structurally related odorant-binding proteins with molecular masses about 18-21 kDa. MUPs are synthesized mainly in liver and excreted through the kidneys with urine. Nowadays MUPs are considered as a key component of the mouse olfactory signature and can provide essential information about the individuality of donors (Novikov et al., 2009; Roberts et al., 2010; Janotova, Stopka, 2011; Kwak et al., 2011). Other physiological functions of the MUPs remain poorly understood. However, there is rapidly growing evidence that MUPs are involved in the regulation of glucose metabolism (Zhou et al., 2009) and can be used as sensitive biomarkers in early diagnosis of carcinogenesis (Chatterji, Borlak, 2007; Ritorto, Borlak, 2011), nephritis (Wenderfer et al., 2009), and parasitic diseases (Manivannan et al., 2010). Using different physiological models, we found that individual MUPs form two distinct combinatorial protein subsets (modules) which appear in both sexes very soon after weaning and resemble genotype-specific «bar-code». The presented data suggest modular principle of the MUPs recognition of chemicals in complex mixtures by selective binding of individual components. Taking into account the recent trends of MUPs application as perspective biomarkers in socially significant diseases, we announce here for the first time the new methodological strategy for creation and development of sensitive biochips based on MUPs 3D matrix.

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Poster session I Poster #181

Role of global lateral inhibition in olfactory bulb network function and odor discrimination in mice

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Granule cells (GC) of the olfactory bulb (OB) can operate different modes of inhibition: recurrent inhibition, local lateral inhibition and global lateral inhibition (GLI). The latter employs Na^+-channel driven action potentials to release GABA from all gemmules thereby inhibiting all connected mitral cells (MC). The role of GLI in OB network function and odor discrimination behaviour is unknown.

We first determined the identity and distribution of voltage-gated Na^+-channel α-subunits in identified GCs of the mouse OB using 3D-Immunohistochemisty (Dondzilo *et al*., 2010). We found that mature GCs exclusively express the $Na_V1.2$ α-subunit. Channels formed clusters at the cell body, dendrites and at the neck of the gemmules. To selectively abolish GLI in GCs, we expressed an shRNA directed against the $Na_V1.2$ α-subunit by stereotaxically delivering AAV1/2-shRNA particles into the OB (Abraham et al., 2010). The effectiveness of shRNA-mediated down regulation of $Na_V1.2$ α-subunit expression was tested in acute brain slices of the mouse OB. Out of four shRNAs targeting different regions of the Na^+-channel mRNA, two abolished the Na^+-current almost entirely and two showed a 50% reduction compared with non-infected GCs. The former two shRNAs effectively abrogated action potential generation in GCs, hence providing a means of selectively testing the role of GLI in odor discrimination. A go/no-go operant conditioning paradigm (Abraham *et al.*, 2004) was used to assess odor discrimination learning, accuracy and time, employing stimuli of different similarity and concentration in mice treated with shRNA, scrambled shRNA and sham-injection. Preliminary results showed that discrimination accuracy was decreased while discrimination time was prolonged when mice discriminated binary odor mixtures at concentrations close to detection threshold. These results suggest a specific role for GLI in discriminating highly similar mixtures at detection threshold.

Poster session I Poster #289

Liking for odor mixture is effected by pre-exposure to its component.

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Many psychological studies have reported the “mere exposure effect”, which states that the more we are exposed to something, the more we come to like it. Some studies suggest that after the experience to a component of an odor mixture, the odor quality of the mixture seems to be similar to the component. Based on this, it is possible that repeated exposure to a component of an odor mixture may lead to increase in liking for that mixture. The aim of this study was to investigate, whether we come to like the odor mixture after mere exposure to a component of it. In experiment 1, an odor quality discrimination task was repeatedly performed to establish pre-exposure to a component. After the task, the liking of the odor mixture as well as the similarity between the mixture and its component was rated. As a result, there was no significant difference between component exposed group and control group (no-pre-exposed to any odors). A cluster analysis on the similarity rating showed that the quality of the odor mixture was enabled to be similar to the pre-exposed component. In experiment 2, participants were exposed to a component of an odor mixture with labels describing the quality of the component odor in order to ensure that participants consistently percept the odor quality. It is expected that the consistent odor perception to the component of odor mixture on each exposure will lead increased liking for the mixture.

Poster session II Poster #104

Contribution of cyclic nucleotide gated (CNG) channel subunits to olfactory adaptation in *Caenorhabditis elegans*

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Adaptation is a fundamental property of sensory systems that enables animals to adjust to ongoing changes in the environment by decreasing their sensitivity to persistent stimulation. In *Caenorhabditis elegans*, the AWC neurons are responsible for sensation of a wide range of attractive volatile odors such as benzaldehyde and isoamyl alcohol (Bargmann et al., 1993). Prolonged odor exposure leads to reversible decreases in the animal's attraction to that odor and this is termed olfactory adaptation (Colbert and Bargmann, 1995; L'Etoile et al., 2002; Kaye et al., 2009). It has been shown previously that the odor specificity of adaptation is determined by the feeding status of the animal (Colbert and Bargmann, 1997). That is, if a well-fed worm is exposed to benzaldehyde for a sustained period, it will adapt to both benzaldehyde and isoamyl alcohol (both sensed by AWC), this process is termed cross adaptation. In contrast, an unfed (starved) worm will adapt to benzaldehyde and its response to isoamyl alcohol will remain intact. The cyclic nucleotide-gated (CNG) channel subunits TAX-4 and TAX-2 are required for AWC-mediated olfactory responses (Coburn and Bargmann, 1996; Komatsu et al., 1999). TAX-4 is an alpha subunit that can form homomeric channels while TAX-2 is a beta subunit that requires TAX-4 to form a functional channel (Coburn and Bargmann, 1996; Komatsu et al., 1999). *C. elegans* encodes two additional predicted alpha subunits, CNG-1 and CNG-3 (Cho et al., 2004a; 2004b; Coburn thesis, 1996). Here we report that CNG-1 is required in AWC to promote short- term cross adaptation between benzaldehyde and isoamyl alcohol. Our results demonstrate that the food- dependent cross adaptation is mediated by an AWC and ASI coincident detection circuit. We also demonstrate that CNG-3 functions in AWC for short-term adaptation responses. To understand the physiology of short-term adaptation we are currently investigating the native assembly of these channels in AWC.

Poster session II Poster #342

Enhancement of amiloride-sensitive (ENaC-dependent) NaCl taste responses in the mouse chorda tympani nerve

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Excess sodium (Na) intake is a serious health concern. Reduction of Na intake can be achieved using enhancers of salt taste. However, there are still no widely acceptable and efficient salt taste enhancers. Recent studies have demonstrated that the NaCl taste reception involves epithelial sodium channel (ENaC). Based on analysis of chemical structures of compounds that enhance salt taste responses in vivo and/or increase ENaC currents in vitro, we have deducted a shared core chemical structure predicted to enhance salt taste through the ENaC-dependent mechanism, synthesized this compound, N-(2-hydroxyethyl)-4-methylpentanamide, and examined its effects on taste responses in the chorda tympani nerve in mice. In C57BL/6J mice, N-(2-hydroxyethyl)-4-methylpentanamide increased the magnitudes of responses to NaCl, but it did not change the responses to KCl and other taste stimuli representing sweet, umami, bitter and sour taste qualities. N-(2-hydroxyethyl)-4-methylpentanamide did not enhance the chorda tympani responses in C57BL/6J mice when NaCl was mixed with amiloride, an ENaC blocker. N-(2-hydroxyethyl)-4-methylpentanamide also had no effect on the NaCl chorda tympani responses in ENaC knockout mice with the ENaCα subunit selectively eliminated in taste tissues. These results suggest that the salt taste enhancement of N-(2-hydroxyethyl)-4-methylpentanamide is specific to Na+ salts and occurs through the amiloride-sensitive (ENaC-dependent) pathway. We conclude that N-(2-hydroxyethyl)-4-methylpentanamide can be used as a lead compound to develop salt taste enhancers, which can be useful for reduction of the Na intake.

Poster session I Poster #343

Influence of flavor preference on the neuronal processing of taste, odor, and flavor

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Preferences have profound impact on subjective perception and evaluation of food. Few studies have, however, explored what impact preferences have on the neural processing of chemosensory stimuli. The aim of the study was to investigate the neural processing of individual preferences toward a beverage in different chemosensory modalities, namely taste, smell, and flavor (odor and taste). To this extent, we used functional magnetic resonance imaging (fMRI) to evaluate brain responses during beverage consumption. A custom-made computer-controlled olfactometer and gustometer were used to present liquid taste and volatile odor stimuli to 34 human volunteers. Participants received the taste, orthonasal odor, and flavor (i.e. retronasal odor and taste) of two familiar drinks (orange juice and beer) and a tasteless and odorless control stimulus. Ratings of perceived pleasantness, familiarity, and intensity were acquired in a mock scanner session. Our preliminary data analyses suggest a negative association between subjective pleasantness ratings of the flavor of the beverage and activations in brain areas previously associated with reward processing, i.e. the anterior cingulate cortex and caudate nucleus, but also areas known to be involved in chemosensory perception, i.e. the insula and adjacent overlying operculum and the piriform cortex. These findings indicate that flavor preference, as assessed by pleasantness ratings, is at least in part regulated by chemosensory brain areas.

Poster session II Poster #344

Skn-1a and taste receptor cell lineage

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In the vertebrate gustatory system, taste receptor cells (TRCs) are assembled to form taste buds. Each TRC is narrowly tuned to one taste modality—sweet, umami, bitter, sour, or salty (at least identified thus far)—and thus taste stimuli evoked by chemicals present in food are discriminated by TRCs at the most peripheral level of the gustatory neural pathways. Continuous turnover of TRCs takes place in the oral cavity, balancing the TRC composition in the taste buds. Maintenance of TRC diversity at the appropriate proportions throughout continuous turnover is required for sensing a variety of different tastes. In the taste buds, a homeodomain transcription factor Skn-1a was specifically expressed in sweet, umami, and bitter TRCs, but not in other types of cells such as sour and amiloride-sensitive salty TRCs. Skn-1a–deficient mice displayed no electrophysiological and behavioral responses to sweet, umami, and bitter stimuli. In Skn-1a–deficient mice, not only the loss of expression of mRNA for sweet, umami, and/or bitter taste–related molecules but also the selective expansion of the expression of mRNA for sour cell molecules were observed. These results demonstrated that Skn-1a is crucial for generating the diversity and balancing the composition of TRCs and shed new light on the transcriptional codes underlying the generation of functional TRC diversity.

Poster session I Poster #105

Axonal transport of a novel isoform of the Insulin/IGF receptor regulates gustatory plasticity in *Caenorhabditis elegans*

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It is of great survival advantage for animals to adapt to their environment by altering their behavior. Recently, we have found that *C. elegans* is attracted to the NaCl concentration at which food has been previously provided, whereas it learns to avoid the past NaCl concentration if it has been starved.

The ASER salt-sensing sensory neuron has a critical role in this gustatory plasticity. In ASER, the insulin/PI3K pathway and CASY-1 act for the avoidance of the NaCl concentration experienced during starvation.

Here, we show that a novel isoform of DAF-2/insulin receptor, which is produced by alternative splicing and named DAF-2c, regulates starvation-induced learning. DAF-2c was localized to the axon of the ASER neuron, whereas DAF-2a, an isoform identified previously, was not. Moreover, the axonal localization of DAF-2c increased in response to starvation. The expression of DAF-2c rescued the learning defect of *daf-2* mutants much more effectively than that of DAF-2a, although both isoforms rescued the dauer-constitutive and long-lived phenotype of *daf-2* mutants.

CASY-1 is the *C. elegans* homolog of Calsyntenins/Alcadeins, which are type I transmembrane proteins highly expressed in mammalian brain. In a suppressor screen of *casy-1* mutants, two missense mutations of *daf-18*, the homolog of PTEN phosphatase that negatively regulates the insulin/PI3K pathway, were identified. Furthermore, the axonal localization of DAF-2c was abolished in *casy-1* mutants. The intracellular domain of CASY-1 physically interacts with a kinesin light chain, KLC-2. The Ras-MAPK pathway modulates the axonal localization of DAF-2c upstream of CASY-1. These data imply that CASY-1 and Kinesin-1 complex regulated by the Ras-MAPK pathway enables starvation-induced gustatory plasticity through the axonal transport of the DAF-2c isoform.

Poster session II Poster #182

Acetylation of histone is involved in the mechanism underlying olfactory learning in young rats

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As one of intriguing epigenetic mechanisms in memory formation and synaptic plasticity, it is well known that histone-associated heterochromatin undergoes changes in structure during the early stages of long-term memory formation. Young rats depend on somatosensory and olfactory function for survival, as they can learn their dam's odor and approach her without visual information. In order to establish olfactory learning, the pairing of odor and somatosensory stimulation is crucial. We have shown that synaptic plasticity in the OB underlies aversive olfactory learning. Our behavioral pharmacological experiments have shown that long-term olfactory memory requires activation of CREB. Western blot analyses have revealed that expression of P-MAPK/ERK is increased for 1 hour after odor-shock training, followed by increase of P-CREB lasting for 6 hours. Epigenetic modifications are recognized to represent a principal interface between intracellular signaling pathways and gene expression. Therefore, we examined whether intrabulbar infusion of trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, facilitates olfactory learning in young rats. TSA infusion during odor-shock training enhanced a conditioned odor aversion in a dose-dependent manner. We further tested whether odor-shock training leads to histone acetylation in the OB and defined the time course of the activation. The acetylation of histone H3 was significantly increased for 1 hour after odor-shock pairing compared with odor only or no stimulation. While the increases of acetyl-histone H4 lasted for 4 hours, total histone H3 and H4 showed no differences among groups. TSA infusion significantly increased histone acetylation levels as well. These results show that histone acetylation is associated with aversive olfactory learning in young rats.

Symposium 7 "Human olfaction" Sunday 24 June

It's about time: Response speed as a key to olfactory perception

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Current theoretical accounts assume radically different unfolding of perceptual aspects of olfaction. Object-centered accounts predict that recognition of unique odor qualities precedes valence decoding. Valence-centered accounts predict the opposite: stimulus-driven valences response precede and guide identification. In two experiments, we used choice-response times to measure processing speed of common odors in different conditions, including decisions regarding odor valence and identity. Odor identity decisions and categorizations were faster and more accurate than decisions regarding odor valence. In fact, valence processing times could be predicted from identification times on an odor-by-odor basis, supporting an object-centered view of odor perception. The results outline a causal framework of how major perceptual features are rapidly extracted from odors.

Contributed talks V "Human olfaction" Monday 25 June

The smell of disease: Changes in human body odor characteristics in response to systemic inflammation

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Ability to detect diseases in conspecifics would be advantageous for the individual. In line with this, rodents avoid body odors of infected individuals. Study 1 indicated that this is possible by way of human smell and human observers. T-shirts from donors (worn for 4 hours) that had received an injection of endotoxin [0.8 ng lipopolysaccharide (LPS) / kg body weight], which causes systemic inflammation, smelled more unpleasant, intense, and sick than shirts from donors that had received a placebo (Saline) injection. GC/MS analysis of the shirts suggested that the change of body odor was not due to a general increase of odorous compounds in the "sick shirts" compared to "placebo shirts" but rather to a qualitative change. Study 2 (ongoing) further investigated the nature of this perception. We compared the body odor of 30 endotoxin (0.6 ng LPS / kg body weight) and 21 placebo (Saline) donors. Again, body odors were sampled during 4 hours using T-shirts. Observers then smelled the shirts and rated intensity, pleasantness, and health. To further characterize the odors, we let them choose verbal descriptors from a set of descriptors developed for body odors. Because the emotion "disgust" has been argued to have evolved for the purpose of disease avoidance, we also assessed observers' disgust proneness and let them rate the feeling of disgust evoked by smells. Results will be discussed in relation to this theory.

Contributed talks VI "Interactions" Monday 25 June

Odor objects from a moth's perspective

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Olfaction requires the extraction of relevant odor information from a highly multidimensional feature space in a dynamic and unpredictable environment. Complex odors can be considered "objects" in much the same way we consider objects as composite images in the visual sense. The insect brain perceives complex odor information using feature extraction at progressive levels within the central nervous system. We propose that these odor features are unique entities separate from their individual volatile components. With *Manduca sexta* as our model species, we have set out to understand how moths perceive olfactory "objects", and how odor features are parsed from sensory input to behavior. Using a variety of techniques including electrophysiology, calcium imaging, and flight tunnel behavior, we have followed the processing of complex host odor information from the antenna to the first level of processing in the CNS, the antennal lobe. We find that *Manduca* detect their olfactory environment using a "selectively non-selective" (A. Anderson) antennal periphery. This sensory input includes neurons both broadly tuned to the majority of plant volatiles, as well as neurons specific to certain plant species. In the antennal lobe, this input is processed non-linearly through the slow temporal patterning of individual neurons, establishing a unique "odor object" percept separate from individual compound identities. Simultaneous imaging between input and output in the antennal lobe confirms that olfactory information undergoes intense network modulation that correlates to behavioral output in wind tunnel experiments. Our findings indicate a stepwise process of feature extraction from sensory fingerprints at the antenna to spatiotemporal modulation in the antennal lobe that establishes a composite image of complex odors even at the first olfactory synapse of the insect brain. This research was supported by EU 6th Framework Programme FET Project iCHEM, the Max Planck Society, the Department of Biotechnology, Gov. of India and the Indian Council of Agricultural Researchand to S.K, and by a Marie Curie IEF-255193 EU Grant to Z. K.

Poster session I Poster #59

Molecular and sensory correlates of host recognition in mosquitoes

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Olfaction is critical in host location and choice by mosquitoes. Differential attractiveness of hosts to mosquitoes has been attributed to variation in chemical and physical cues. Genome information has enabled molecular characterization of odorant receptors of *Anopheles gambiae* in heterologous expression systems. Through combined bioinformatical, molecular, physiological, chemical and behavioral analyses we aim to identify novel natural ligands from hosts and non-hosts with potential use in the chemical ecological management of this disease vector mosquito. To reach this goal we have characterized the peripheral olfactory system of *An. gambiae* using single sensillum recording (SSR) analysis, and the response of transgenically expressed *An. gambiae* odorant receptors to complex ecologically relevant odors (host, non-host, floral and host environment odors), by SSR and combined gas chromatography-SSR analyses. The tuning of the transgenic ORs reflects relevance to ecologically important signals, but does not fully account for the responses observed ORNs *in vivo*. Thus, the tuning of the olfactory receptor neurons is possibly achieved by additional mechanisms than the receptor expression alone. We are therefore using information from available genome sequencing projects to investigate the structure, evolution and expression characteristics of olfactory genes and their influence on receptor function. These studies will improve our understanding of the role of the peripheral olfactory system in host finding in *An. gambiae*.

Poster session I Poster #345

Regional differences in gustatory responses to bitter substances between the soft palate and fungiform papillae

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To clarify the regional differences in gustatory responses to bitter substances between soft palate (SP) and fungiform (FF) taste buds, we examined the co-expression of Gα-gustducin with taste receptors, the impact of Gα-gustducin knock-out (gKO) on neural responses to bitter compounds, and behavioral changes caused by gKO. Gα-gustducin was expressed in 87% and 88% of Tas2rs-cells in the SP and FF, respectively. In spite of significant colocalization of Gα-gustducin and bitter taste receptors, there were no significant differences in the chorda tympani (CT) responses to both quinine-HCl (QHCl) and denatonium (Den) between gKO and wild type (WT) mice. In contrast, GSP responses to these compounds were markedly reduced in gKO mice with an apparent elevation of thresholds (>10-fold). These results suggest that: 1) Gα-gustducin plays a critical role for bitter taste transduction in the SP, while not in the FF, 2) other Gα-subunits co-expressed with Gα-gustducin in the FF are sufficient for responses to QHCl and Den, and 3) robust GSP responses to QHCl and Den occur in the SP by a Gα-gustducin-dependent mechanism which is absent in the FF. Further single fiber analysis for neural responses to bitter substances from the GSP and CT in GKO and WT mice may provide better understanding of the mechanisms for bitter taste transduction.

Poster session II Poster #438

Aversion- and fear-inducing properties of wolf urine

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Wolf urine contains many volatile constituents that may be used for chemical communication. The levels of certain urinary volatiles vary widely in the three wolf urine samples which acquired in the different periods. To identify the volatiles in wolf urine that induce avoidance and freezing behavior to female mice, behavioral, chemical and immunohistochemical studies were performed. We found that one of three wolf urine have higher levels of specific volatile chemicals than the other urine samples. In addition, this wolf urine sample induces freezing behavior and avoidance behavior most effectively of all three wolf urine samples. Moreover, only this wolf urine sample caused pronounced activation of Fos in the accessory olfactory bulb (AOB). When these specific chemicals which observed from the active urine sample confront to the mice, the fear-inducing behavior and activation of Fos in AOB to female mice was markedly enhanced. Our results suggested that this fear-induction is due to increased wolf urinary chemosignaling.

Contributed talks II "Gustation" Monday 25 June

Starvation-induced elevation of taste responsiveness and expression of a sugar taste receptor gene in a wild-derived strain of *Drosophila melanogaster*.

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Animals including human beings increase their feeding motivation under starved condition. Such a starvation-induced increase of feeding motivation might different among wild-derived strains in *Drosophila melanogaster*. In the behavioral experiments using two wild-derived *Drosophila* strains, Mel6 and TW1, we found that TW1, rather than Mel6, drastically decreased the feeding threshold to sucrose after 24 h starvation. Thus, the starved TW1 preferably ingested low concentration of sucrose such as 0.1 mM to water. At that time, TW1 showed significant elevation of taste responsiveness to low concentrations of sucrose and gene expression of a sugar receptor molecule for sucrose, Gr64a, in the sugar receptor neuron. Mel6 and TW1 flies show a good survivorship under an appropriate dietary condition, but both died out within a few days of starvation. However, when they were provided with a less nutritious food (10 mM sucrose), TW1 survived longer than Mel6. In case of TW1, the starvation-induced decrease in the behavioral and the sensory thresholds could advantageously work in searching and utilizing less nutritious foods. Wild-derived strains may have their particular survival strategies for starvation or sub-optimal nutrient period. Here, we proposed the starvation-induced functional change in the taste sensory system as one of such strategies.

Poster session I Poster #249

Olfactory function loss in ESRD patients with sleep disorders

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Dialysis-related sleep disorders are a common complication of end-stage renal disease (ESRD) (80%), including obstructive or central sleep apnea (OSA/CSA). Despite its prevalence, the precise etiology and pathogenesis of this disorder remains unknown (Zoccali, Mallamaci, & Tripepi, 2001). ESRD patients have also been found to have impaired olfactory discrimination (Frasnelli et al., 2002). Because of the high prevalence of sleep disorders and impaired olfaction within the ESRD population, we questioned whether patients with dialysis related sleep disorders would exacerbate impaired olfaction.

Using the University of Pennsylvania Smell Identification Test (UPSIT) we measured olfactory discrimination in 11 ESRD patients who were tested for dialysis related sleep disorders. UPSIT norms were used to determine percentiles. Of the 11 patients, 91% demonstrated microsmia or anosmia. None of these 11 patients scored above the 36 percentile indicating uniformly poor olfactory performance. This pilot study forms a base for future studies on olfactory impairment in ESRD patients.

Poster session II Poster #196

Retinoic acid receptor and CNGA2 channel signaling are part of a regulatory feedback loop controlling axonal convergence and survival of olfactory sensory neurons

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Little is known about the identities and functions of extracellular signaling molecules that work in concert with neuronal activity to regulate refinement and maintenance of the mouse olfactory sensory map. We show that expression of a dominant negative retinoic acid receptor (RAR) in olfactory sensory neurons (OSNs) increased the number of glomeruli that incorrectly contained OSN axons expressing different odorant receptors. This phenotype became apparent postnatally, coincided with increased cell death, and was preceded by increased Neuropilin-1 and reduced Kirrel-2 expressions. Kirrel-2-mediated cell adhesion influences odorant receptor-specific axonal convergence and is regulated by odorant receptor-signaling via the olfactory cyclic nucleotide-gated (CNG) ion channel. Accordingly, we found that inhibited RAR function correlated with reduced CNG channel expression. Naris occlusion experiments and analysis of CNG channel-deficient mice further indicated that RAR-regulated CNG channel levels influenced the intrinsic neuronal activity required for cell survival in the absence of odor stimulation. Finally, we showed that CNG channel activity regulated expression of the retinoic acid-degrading enzyme Cyp26B1. Combined, these results identify a novel homeostatic feedback mechanism involving retinoic acid metabolism and CNG channel activity, which influences glomerular homogeneity and maintenance of precisely connected OSNs.

Poster session II Poster #418

Bioelectronic odor detection

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The European project BOND (Bioelectronic Olfactory Neuron Device, PCRD 7, http://bondproject.org/) aims at using olfactory receptors (ORs) carried by nanoscale liposomes as sensing elements of an electrochemical array, for specifically detecting target odorants. This project integrates bio, micro/nano, and information technologies into a bioelectronic nanoplatform, benefiting the ORs high sensitivity to detect low concentrations of target odorant molecules, possibly from a complex odorant mixture.

In the natural olfactory system, a large number of ORs exhibit various levels of specificity and sensitivity to odorants. Here, the ORs relevant to monitor the presence / concentration of a target odorant are identified by single-cell RT-PCR on neurons exhibiting a calcic response to this odorant. 3D model structures of these ORs can then be produced using a dedicated program, and odorants docked thereon, to rank them relative to their binding efficiency. These ORs are then expressed in yeasts, and liposomes of nanometric scale, carrying the ORs on their lipid bilayer, are prepared from yeast membrane fraction. Surface Plasmon Resonance is used to monitor the ORs response to the target odorant.

The active part of the bioelectronic sensor consists of a silicon nanoelectrode with an electrochemical cell and a dedicated electronic chip. To provide the sensor with high sensitivity, gold nanoparticles are generated on the gold surface by electrochemical chronoamperometry to favor nucleation, to increase the nanotransducers surface and amplify the electrochemical transduction signal. Nanosomes carrying ORs are immobilized on electrodes using cmyc or 6HIS tags. Gold nanoparticles induce a surface area increase measured by both Electrochemical Impedance Spectroscopy and Atomic Force Microscopy. Efficiency of nanosomes immobilization, amplified in the presence of gold nanoparticles, and ORs functional response, are monitored through the charge transfer resistance measured by EIS.

Poster session II Poster #376

Sensory perception of sodium chloride and its substitutes

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Sodium chloride is used as the main source for salty taste. Its content in food has been more observed in recent years, because high consumption of sodium is connected with hypertension and other diseases. The World Health Organization (WHO) has recommended to decrease its level in food and therefore the members of Federation of the Food and Drink Industries of the Czech Republic (FFDI) have accepted in 2009 voluntary agreement about decreasing sodium level in food or trying to use sodium substitutes**.** Different substitutes of salt: light salt Mary (Czech product), No Salt, Kardisal and Lacto salt optitaste have been sensory tested. It is important to select substitute ingredients with a taste profile that best corresponds to the food. The aims of the study were to determine for substitutes the same concentration as standard sodium chloride, compare sensory perception by population related to salt intensity, identify the just-noticeable difference in concentration at which consumers noticed a decrease in salty taste in food products. The influence on the food properties, special for bakery products, was also studied. The sensory evaluation were done with panel of trained assessors and the results were compared with consumer panel.

Poster session I Poster #183

Broad sensory-induced activity rapidly alters the intrabulbar map

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In mammals, each olfactory bulb contains mirror-symmetric glomerular maps that are organized to reflect odorant receptor (OR) identity. Within these maps glomeruli that receive axons from olfactory sensory neurons (OSNs) expressing the same OR are referred to as iso-functional glomeruli. Previously we revealed that iso-functional glomeruli are specifically linked to one another through a set of intrabulbar projections (IBPs) mediated by tufted cells giving rise to an intrabulbar map. We demonstrated that this map develops postnatally through a process of activity-dependent refinement. We also showed that IBPs exhibit extensive anatomical plasticity throughout adulthood such that loss of odorant-induced activity through naris closure causes broadening of projections while reintroducing odorant activity restores projection specificity. Since sensory deprivation can also affect the survival of olfactory bulb neurons, we sought to determine whether odorant-induced activity is playing a permissive or instructive role in shaping IBPs. To address this we used UBI7 mutant mice in which the rat I7 (rI7) receptor is broadly expressed in all mature OSNs. Since rI7 is expressed at low levels in UBI7 mice it does not block expression of endogenous ORs and does not alter the glomerular map. We show that upon daily exposure of UBI7 mice to octanal, a ligand for the rI7 receptor, the intrabulbar map is quickly disrupted exhibiting significant broadening of IBPs within just one week while the glomerular map remains intact. Furthermore, we show that removal of octanal from previously exposed mice causes IBPs to re-refined to control levels of specificity. Importantly, we saw no indication of the large neural loss as typically associated with sensory deprivation suggesting IBP plasticity is not driven by neural loss. Thus, we propose that activity plays an instructive role in IBP refinement and that changes in activity patterns can dynamically alter their specificity.

Contributed talks IV "Olfactory receptors, ligand interactions and transduction mechanisms" Monday 25 June

Exploring insect odorant receptor function through the use of novel chemical modulators

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The detection of olfactory cues from the environment is largely mediated through large families of odorant receptors (ORs). While ORs in many animals act as GPCRs, insect ORs function as odorant-gated ion channels. Here, a functional OR complex consists of a conventional OR and an extraordinarily conserved co-receptor, Orco, in an unknown stoichiometry.

A novel Orco agonist, VUAA1, has recently been identified and utilized to investigate the mechanistic properties of insect OR function and demonstrate that Orco subunits from various insect orders can form functional homomeric channels. VUAA1 was also used to examine the influence of conventional ORs on the ion channel pore, where apparent differences in ion permeability and susceptibility to channel blockade were observed. The exploration of the chemical space around VUAA1 yielded several analogs, and one of which not only acts as a competitive antagonist to VUAA1, but also as an allosteric, noncompetitive antagonist to odorant-mediated activation. Through the continued use and advancement of this novel class of modulators, we can continue to gain more insight into the structure-function relationship of insect ORs, as well as potentially develop chemical deterrents against disease vectors and agricultural pests.

Poster session II Poster #90

Evolutionary pattern of the AmOR11 orthologs

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The 9-oxo-2-decenoic acid (9-ODA) is the main component of the Queen substance (QRP) of the honeybee (*Apis mellifera* L.). QRP and 9-ODA are two likely keys of the queen's dominance on the hive workers and consequently one of the central components of the awesome social organization of honeybee. Such high level of social organization is more broadly observed in the family Apidae. Besides the ubiquitous *A.mellifera*, the genus *Apis* L. contains 6 other species displaying similar levels of sociality. Likewise at the family level, the corbiculate Apidae are grouping together Apini (*Apis*) and three other tribes: Bombini (Bumblebees), Euglossini (orchid bees) and Meliponini (stingless bees), with comparable level of social organization (from parasocial in Euglossini to advanced eusocial in Meliponini). In Apini, Bombini and Meliponini, the division of labor within the colony implies a division in castes, queen, males and workers, which are morphologically readily identifiable. In honeybees (Apini), it is known that the social domination of the queen on the workers relies largely on a physiological pathway involving 9-ODA (in the QRP) and its activation of the olfactory receptor AmOR11 (characterized by Wanner and co-authors). Since 9-ODA has been characterized in the queen pheromonal bouquets of 4 amongst the 7 species of *Apis* and that all the species in that genus are eusocial, we hypothesize that AmOR11 could be evolutionary conserved. We studied the evolutionary pattern of AmOR11 within (i) Apis and (ii) the corbiculate Apidae. Phylogenetic topologies are inferred based on the sequences of the AmOR11 orthologs using distance, Bayesian and maximum likelihood approach. The pattern of evolution of the AmOR11 orthologs is discussed in terms of trait evolution, in regard of the sociality status of the concerned taxa. This analysis is further used to estimate the phylogenetic signal contained in the studied receptor genes.

Poster session I Poster #167

Odor coding beyond the first sniff: adaptation and memory in the olfactory bulb

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Research on olfactory coding has focused on how odors are represented during the breathing cycle, but less attention has been paid to how these representations shift between breaths. We recorded from the olfactory bulb of awake, head-fixed mice, and analyzed how the olfactory code evolved during odor presentation and afterward. Some individual cells' odor code change between the first and subsequent sniffs. On the population level, the population representation for the first sniff is significantly different from subsequent sniffs, but settles over time. Moving beyond the time of odor presentation, a subset of cells respond following the cessation of odor. Using a prediction algorithm, we show that these post-odor breaths contain odor- and concentration-specific information, a form of short-term memory. When thinking about the odor-response, one must be careful to consider these dynamics over time.

Symposium 3 "Chemosensory receptors in non-chemosensory tissues" Saturday 23 June

Olfactory receptors and regulation of muscle stem cell behavior

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Adult skeletal muscle has an extensive capacity for regeneration due to the presence of muscle stem cells. Multiple factors are required for successful muscle regeneration after injury. We show that the expression of multiple olfactory receptors is induced during muscle regeneration in mice. One of these, MOR23, regulates migration and adhesion of muscle stem cells and is necessary for proper skeletal muscle regeneration. In the absence of MOR23, aberrant regenerated muscle fibers are formed similar to those seem in various muscular dystrophies. We are determining the downstream effector molecules that mediate MOR23 action in skeletal muscle. These data suggest that modulation of olfactory receptor signaling in muscle may be beneficial in enhancing muscle repair in muscular dystrophies.

Symposium 6 "Robotics and artificial chemosensors" Sunday 24 June

New-generation coupled technologies for infochemical communication

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The enormous diversity of molecules in the natural world is the *lingua franca* of the biological domain. Insects, for instance, use specific ratios of pheromones as both hetero- and conspecific cues to mediate predator avoidance, mate location and metabolic processes. Whilst the electron may be the fundamental unit of information within technology, we have built a set of coupled molecular control and detection technologies for mediating a new form of communication *via* ratios of infochemicals. Here we describe an experimental set-up demonstrating such infochemical communication within naturally turbulent plume conditions. Presynthesised ligands are delivered into the world in controlled ratios by a "female" signalling mobile robot platform (termed chemoemitter) hosting a micromachined "artficial gland" based upon controlled pheromone release of female moths. Ratiometric information in the world is recovered by a complementary and active-search "male" robot platform (termed chemoreceiver) equipped with an array of polymer-coated SAW chemosensors driving a real-time neuromorhphic model of the first stage of chemosensory processing in insects, the antennal lobe (AL). We will show how the dynamical trajectories of the AL neuronal population encodes specific ratios of chemical signals in time, thus achieving ratiometric infochemical communication. Our research paves the way for next-generation microscale and high-bandwidth coupled chemical sensing and molecular control technologies, providing

unprecedented opportunities in medical, environmental, and manufacturing contexts.

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Poster session II Poster #184

Adult neurogenesis in the accessory olfactory bulb of female mice is critical for mate recognition

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Olfactory cues in rodents regulate multiple aspects of social/sexual interactions. Recent data indicate that a continuous supply of pools of newborn neurons during adulthood provides a preferential cellular substrate to track and record environmental stimuli. Here, we have investigated whether adult neurogenesis in the olfactory system of female mice can play a role in pheromone-mediated mate recognition. By using the well-known model of mating-induced imprinting (Bruce-effect), we demonstrate that this olfactory memory formation critically depends on the presence of newborn granule neurons in the accessory olfactory bulb. Accordingly, we show that, in adult female mice, exposure to male pheromones increases the number of new granule cells surviving in the accessory olfactory bulb. This neuronal addition depends on the detection of sensory cues by the vomeronasal organ and requires centrifugal feedback activity from the amygdala. The stimuli affecting neuronal survival are contained in the low molecular weight fraction of urine and are implied in pheromonal recognition during mating. By chemical depletion of newly generated bulbar interneurons, we prove a direct role of renewed granule cells in the accessory olfactory bulb in preventing pregnancy block by mating male odours. Taken together, our results indicate that adult neurogenesis is essential for specific brain functions such as persistent odour learning and mate recognition.

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Poster session I Poster #439

An odor-dispensing device for the treatment of sleep apnea

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Obstructive sleep apnea (OSA) is a prevalent sleep disorder characterized by repetitive cessation or decreased amplitude of breathing lasting 10 s or more. Clinical consequences cover a wide spectrum including cardiovascular, neurocognitive, and metabolic dysfunction. Current standard of care is continuous positive airway pressure (CPAP), which is effective but characterized by low compliance due to encumbrance. Studies of olfactory processing during sleep suggest that pure olfactory and mildly trigeminal odorants do not arouse or wake, but nevertheless induce a sniff response. With this in mind we set out to test the hypothesis that odors can be used to "jump start" respiration during OSA without waking. We developed a computer-controlled touch-screen-interfaced home device that delivers pulses of odorants at either fixed intervals, or in response to apnea. Using an array of sensors, the device monitors and records nasal and oral respiration, heart rate, oxygen saturation, and snoring. Overall activity and light conditions are monitored by wrist-worn actigraph.

The device obtains daily subjective measures of sleep quality through an interactive touch-screen, an Epworth sleepiness questionnaire, as well as an objective measure derived from a touch-screen-interfaced measure of reaction time. The device is placed in the participant's home for 2 weeks, recording data for 3 consecutive nights in each week, one week with odors and one week without (counter-balanced, participants uninformed to order). We used 5 odorants, which were delivered in pulses of 15 s, both in a random order once every 13 minutes, and automatically in response to an apnea. To date, we have studied 3 individuals. This sample is too small for statistical analysis, but descriptively: In 2 out of the 3 subjects, odor administration improved subjective sleep quality, snoring, oxygenation, and RDI. Reaction time improved in all 3. Data from a larger sample will be presented in this presentation.

Poster session II Poster #304

Determinants of age-related olfactory loss (Presbyosmia): insights from the Rush Memory and Aging Project

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Presbyosmia is an important global health problem affecting critical human functions with growing clinical impact as societies age. We characterized factors that underlie susceptibility to presbyosmia using data from the Rush Memory and Aging Project (MAP). MAP collected extensive health information by standardized interview on older subjects from retirement communities throughout northwest, Illinois, USA, who were without dementia at baseline, including social information, demographics, health history, and 24 biomeasures including olfaction assessed by use of the Brief Smell Identification test (n=1200, 7.8%African American, 6.3% Hispanic).

We performed linear regression with the number of correctly identified odors as the dependent variable. As with previous reports, olfactory performance was inversely related to age ($P<0.001$) and females had better performance than males ($P<0.001$). We then adjusted our analyses for these variables. There was no significant association of head injury, heart disease, cancer, diabetes, or history of smoking ($P>0.05$, all). Elevated symptoms of depression were associated with decreased olfaction ($P=0.009$) as was cognitive impairment ($P<0.001$). Interestingly, we found worse olfactory performance among African-Americans and Hispanics compared to non-Hispanic whites ($P=0.049$) and higher scores among those with increased income ($P<0.001$). Results using ordered logistic regression were similar.

These data suggest that race/socioeconomic status affect presbyosmia, providing support for the concept of genetic and/or environmental modifiers for this condition. Study of available genetic and pathologic data in MAP will help delineate these possibilities and will provide additional insights into this prevalent and burdensome form of sensory loss.

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Poster session I Poster #407

GWAS in 3 isolated populations identifies a gene for PROP sensitivity.

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PROP sensitivity is one of the most studied taste-related traits. It is largely dependent on the TAS2R38 genotype, and it has been clearly shown that people with the AVI/AVI genotype are mostly insensitive to PROP. Despite its clear genetic background, TAS2R38 explains only part of the PROP sensitivity variance. To investigate if further genetic variants could explain PROP tasting, we measured PROP responsiveness in 3 isolated populations in Italy withthe LMS scale. Association analysis was conducted on PROP intensity corrected for NaCl intensity, sex, age and TAS2R38 genotype. The most significant hit was on rs2241314, very close to the GHRL and GHRLOS genes. The first gene encodes for the ghrelin protein, and the other one to its antisense. Recently it has been shown that PTC gavage augments ghrelin levels in the blood and decreases food intake in mice via the g-protein α-gustducin (Janssen et al 2011). It could be that ghrelin expression and levels could influence PROP tasting (or phenotype) through a negative feedback mechanism, however further studies are needed to clarify this relationship.

Poster session II Poster #279

Electro-olfactorgram recording (EOG) from the human olfactory epithelium during natural nasal respiration

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The olfactory system presents a unique opportunity to directly record neural activity in humans in vivo. Olfactory receptor neurons (ORNs), a form of PNS-CNS transition neuron outside the skull, located in the olfactory epithelium, enable in vivo recording of olfactory responses from awake behaving humans. Till now, the work frame for EOG research was recording during short respiration cessation, so as to avoid the ORNs response to the airflow overshadowing the olfactory evoked response. Because the sniff is an integral part of the olfactory perception, its absence may alter or even abolish the ORNs response. Here we set out to develop a method for EOG recording during natural human nasal respiration. EOGs were recorded at 1 kHz from a normosmic healthy subject (M, age = 25) using an Ag/AgCl electrode coated with Teflon tubing (0.8 mm OD) filled with Ringer-agar (1%). Using a computer-controlled olfactometer we delivered an odorant (Ethyl 3-methyl-3-phenylglycidate, CAS 77-83-8) into the recorded nostril via Teflon tubing (inner diameter = 2.15 mm), maintaining steady mechanical and thermal conditions (5.5 SLPM, 37 °C, 80% RH). Stimulus duration was 0.5 s, and inter-stimulus-interval was 25 s. The experimental paradigm contained four blocks; each consisted of five-odorant presentations during respiration cessation and five presentations during natural nasal respiration. We found that simple high-pass filtering with a corner frequency of 1 Hz enabled on-line visualization of the EOG recorded during natural nasal breathing. Moreover, a more advanced signal processing method almost completely reconstructs the olfactory portion of the recorded EOG, which enables the recording of in vivo ORN activity and perception simultaneously in naturally breathing humans.

Poster session II Poster #218

Channel properties of the splice variants of the olfactory calcium-activated chloride channel – Anoctamin 2

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Anoctamin 2 (ANO2) is the olfactory calcium-activated chloride channel that amplifies the odorant- evoked receptor potential in olfactory receptor neurons (Stephan et al., 2009). Four different splice variants of ANO2 have been identified in olfactory mucosa – Two predominant variants containing Exon 4 (which encodes 33 amino acids in the predicted intracellular N-terminal region) but differing in their transcription initiation sites, and two minor variants which lack Exon 4 and having different transcription initiation sites. To understand the functional significance and biophysical properties of these splice variants, we expressed them individually in HEK293t cells and investigated their channel properties using inside-out patch clamp electrophysiology. The ANO2 variants containing Exon 4 displayed current properties that are largely similar to those of the native calcium-activated chloride channel. However, the Exon 4-lacking variants failed to generate any recordable currents in response to Ca^{2+} stimulation. Among the two Exon 4-containing ANO2 variants, we observed a difference in Ca^{2+} sensitivity presumably due to their differing N-terminal ends. We propose that the Exon 4 domain is indispensable for generation of ANO2 channel currents, and that the N-terminal sequence may have a role in determining the channel sensitivity to Ca^{2+}.

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Poster session II Poster #166

Assessment of activation of olfactory and vomeronasal sensory neurons by a preputial gland derived compound, farnesol: A calcium imaging study

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Pheromones are potent molecular signals that are fundamental for species-specific chemical communication, thus organizing a wide range of social behaviours such as finding and identifying a mate, regulating the level of aggression and dominance, and mediating the recognition of kin and non-kin. The olfactory receptor neurons (ORNs) in main olfactory system and vomeronasl sensory neurons (VSNs) of accessory olfactory systems are responsible for mediating pheromone information in rats. Earlier we have identified the compound, farnesol, as testosterone dependent compound in preputial gland and putative pheromone in male rat. In the present study, activation of isolated ORNs and VSNs of both male and female rat to the preputial gland derived compound, farnesol was determined by calcium imaging analysis. Among the tested neurons, 30 % of ORNs and 18 % of VSNs of female rat were responded to farnesol by exhibiting the intracellular calcium response. Similarly, 31% of ORNs and 29% of VSNs of male rat were responded. The results indicate that farnesol do have pheromonal property and can interact with some types of receptors expressed in ORN and VSN of both male and female rat for manifesting the behavioural responses involved in social and sexual interactions. The study concluded that the olfactory-vomeronasal system of rat may play a synergistic role with irrespective of sex for detecting farnesol.

Poster session I Poster #441

Olfactory evidence: A new era for odor profiling in birds

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We have developed an efficient and reproducible analytical means of studying human scent samples for proof of concept of human scent as a biometric measure in law enforcement applications. We are using this forensic approach to investigate individual odor recognition in birds, using Leach's storm petrels (*Oceanodroma leucorhoa*) as our avian model. Although both adult and young birds recognize personal odors, the chemical makeup of individual odor signatures is still in question. We are therefore evaluating chemical profiles from thirty nine adult feathers and twenty six burrows (triplicate samples of each) using instrumental analysis borrowed from forensics. The technique allowed the discrimination of nest and feather odors using Solid Phase Microextraction coupled with Gas chromatography/Mass spectrometry (SPME-GC/MS). The identification of commonly occurring compounds such as pristane, nonanal, cyclododecane, toluene, and nonanoic acid has been achieved on over 80% of the samples. Primary odor constituents have allowed us to test whether we can find sex and/or genotypic traits rooted in the odor volatiles emanating from these biological matrices. Using a subset of chemical compounds, from multiple samples taken per individual, allowed our technique to further corroborate the importance of an intra-subject odor baseline for chemical odor discrimination. Analysis comparing similarities between burrow and feather odor is currently underway.

Poster session II Poster #290

The effect of age on perception of large and small odor molecules

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Objective Decrease in olfactory function during the course of life has been well documented in many studies. This study examined the effect of age on perception of large and small odor molecules.

Methods A total of 142 volunteers (48 males, 94 females) were divided into two age groups. Group 1 comprised 50, group 2 92 subjects (mean age 25 or 81 years, respectively). Olfactory threshold and identification were obtained using "Sniffin'Sticks"; in addition, we examined thresholds of cinnamaldehyde, limonene, farnesol, and bisabolol. The odors cinnamaldehyde and limonene represented small molecules (MW < 137 g/M); farnesol and bisabolol large ones (MW >222 g/M). Using two validated questionnaires the subjects of group 2 were asked about their mental state and the significance of olfaction.

Results Compared to the young subjects older people were less sensitive in terms of odor identification and odor thresholds of small and large molecules. In older subjects odor thresholds of small and large molecules differed significantly ($p< 0.01$) with reduced sensitivity to farnesol and bisabolol, whereas in younger subjects no such difference was observed.

Discussion It appeared that during the course of life sensitivity for large odor molecules decreased more than the sensitivity for small molecules. An explanation could be that large molecules bind more specifically to olfactory receptors, whereas small molecules can bind - more unspecifically - to a number of receptors. Because the capability of regeneration of the olfactory epithelium probably declines with age, it is possible that the number of specific olfactory receptors and therewith the sensitivity for large odor molecules also decrease.

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Poster session II Poster #38

Interference of moth pheromone detection by plant-derived odorants

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Male moths detect female-released sex pheromone blends by specialized olfactory sensory neurons (OSNs), housed in sensilla hairs on the antennae. OSNs for different pheromone components are endowed with specific pheromone receptors in their dendritic membrane and converge their axons into separate compartments of the macroglomerular complex (MGC), the pheromone-processing center within the antennal lobe (AL). Previously we have found that in Heliothis virescens the detection of the major sex pheromone component (Z)-11-hexadecenal (Z11-16:AL) involves the pheromone receptor HR13 and the pheromone binding protein PBP2. Recent work on H. virescens has indicated that plant-derived odorants co-existing in the environment of calling females may interfere with the detection of pheromones. Therefore, to scrutinize an interference of odorants with molecular elements of pheromone detection, we have analyzed Z11-16:AL binding to PBP2 and HR13 in the presence of plant-derived odorants. Using a 1-NPN based competitive fluorescence binding assay odorants were found not to bind to PBP2 or interfere with Z11-16:AL binding. In contrast, calcium imaging experiments with a HR13-expressing cell line revealed inhibitory effects of odorants on the pheromone-induced cell responses, indicating that odorants interfere with Z11-16:AL detection at the pheromone receptor level. To approach the question if a peripheral interference between pheromones and odorants at the OSN level may have consequences for the processing in the brain, in vivo imaging experiments were performed monitoring the activity in the MGC and odorant-processing ordinary glomeruli in the AL. The results show that co-stimulation of male antenna with Z11-16:AL and certain odorants decreases the pheromone-induced activity in the cumulus region of the MGC, where Z11-16:AL-specific OSN terminate. This work was supported by the Deutsche Forschungsgemeinschaft SPP 1392.

Poster session II Poster #96

Effect of mating experience on the host plant preference of female and male adults of the polyphagous moth, *Spodoptera littoralis*

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For herbivorous insects of both sexes, selecting the correct host plant is a crucial decision. For the female, it has a direct consequence on her offspring fitness whilst for the male, it could be linked to the presence of a mate. For polyphagous species, which are able to use a broad range of possible hosts, host plant choice is complex with respect to innate and/or earlier experience. Here for both sexes of the moth Spodoptera littoralis we have investigated the effect of previous experience involving cues associated with mating on subsequent host preference. We examined male attraction to pheromone in a plant odour background and female oviposition. The mating experience on a specific plant increased both female and male preference for this plant in subsequent behaviours. For polyphagous insects, combining previous host experience together with innate plant preference could represent an advantage to allow discrimination within a broad range of host plants, and also facilitate colonisation of novel environments. Furthermore, it can reduce deleterious costs when detecting and assessing host plants.

Poster session I Poster #219

Role of the sodium channel Nav1.7 in accessory olfactory system function

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We recently showed that the voltage-gated sodium channel Nav1.7 functions as a key element in odor perception. Removal of Nav1.7 from all olfactory sensory neurons (OSNs) by means of the Cre-loxP technique causes congenital general anosmia due to a lack of Nav1.7 in axons and presynaptic OSN boutons, leading to a loss of synaptic transfer in glomeruli of the main olfactory bulb. The mouse olfactory system comprises multiple subsystems that employ distinct signal transduction mechanisms, but it is unknown whether each subsystem has evolved a unique subset of sodium channels or whether the same channels underlie action potential generation and conduction in all subsystems. We have begun to address this question by focusing on the accessory olfactory system. We find that conditional Nav1.7 mutants lack typical VNO-dependent social behaviors such as intermale aggression and interspecies defensive behaviors, including risk assessment and avoidance behavior. These deficits are at least partially due to a lack of vomeronasal sampling caused by the anosmia phenotype of the Nav1.7 mutants, pointing to an important interaction between the main and accessory olfactory systems in the regulation of social behaviors. We also find that Nav1.7 is strongly expressed in vomeronasal sensory neurons and their axons, especially in glomeruli of the accessory olfactory bulb, suggesting that Nav1.7 could play a *bona fide* role in action potential transmission of vomeronasal nerves. Experiments are underway to determine the precise function of Nav1.7 in vomeronasal signaling. Taken together, our findings suggest that Nav1.7 could play a critical role in the control of neural activation of both main and accessory olfactory systems.

Poster session II Poster #250

Human olfactory instrumental conditioning

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Olfactory cues are commonly used in animal paradigms of instrumental conditioning, a well-established model of learning and decision-making. Whether olfactory cues are equally effective in humans has not been well-characterized. The use of odor holds an important advantage: the same limbic brain regions underlying emotional learning share considerable anatomical overlap with the olfactory system, including key regions of the medial temporal and basal frontal lobes. It thus follows that olfactory models of associative learning should provide keen insights into the neurobiology of adaptive behavior. In a pilot study of 25 subjects, we assessed learning using a novel olfactory instrumental conditioning paradigm: two different odors were used as conditioned cues, which were delivered to subjects on separate trials using a computer-controlled olfactometer. A third odor was used as a learning-independent control cue. Upon odor delivery, subjects were asked to make one of two button responses associated with either a higher (60% or 70%) or lower (40% or 30%) probability of winning or losing money. Through trial-and-error, subjects learned the odor-response contingencies that would maximize gains and decrease losses. These behavioral findings are among the first to demonstrate olfactory instrumental conditioning in humans, and future work will combine this paradigm with event-related functional MRI to examine the neural substrates of these effects.

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Symposium 5 "Interspecific chemointeractions" Sunday 24 June

Humidifying the black box of environmental context in insect foraging and orientation

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Environmental cues such as relative humidity (RH) are ubiquitous features of terrestrial habitats, yet few studies have explicitly documented how RH impacts chemically mediated insect – plant interactions. For large hovering insects such as hawkmoths (Lepidoptera: Sphingidae), which fly long distances in search of mates, host plants and floral nectar, RH could be relevant at several spatial scales and ecological contexts. Hawkmoths are known to disperse from dry to humid habitats, and live longer and take larger nectar meals in a humid environment. At the smallest scales, evening primrose (*Oenothera cespitosa*; Onagraceae) flowers produce transient humidity plumes (c. 5% RH above ambient) which accurately indicate nectar presence in newly-opening flowers. *Hyles lineata* moths distinguish between artificial flowers showing this scale of RH difference in binary choice flight cage assays, showing first-visit preferences for artificial flowers with elevated RH over those with ambient RH, in the absence of a nectar reward. *Manduca sexta* moths show similar responses to 5-10% RH differences in binary choice wind tunnel assays. These preferences disappear when nectar rewards are comparable in checkerboard (but not homogeneous) floral arrays, and when background RH is high (above 40%). We discuss the relevance of RH to foraging hawkmoths as an indicator of floral profitability, appropriate habitat and distance orientation.

Symposium 22 "Odor memory and perception: cells to circuits" Wednesday 27 June

Memories for odor experience: Mapping circuits in the rat through multi-site recordings of oscillatory activities.

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Perception and memory are distributed and dynamic processes. They rely on a large variety of circuits involving functionally specialized brain areas. If we accept this concept, we have to face the question of how the brain could select and coordinate such distributed activities to produce a unified and adapted cognitive activity. One alternative proposed (reviewed in Varela et al, *Nat. Neurosci.*, 2001) is the formation of large-scale assemblies of neurons, transiently linked by reciprocal dynamic connections. These assemblies could be formed within a given area, between structures situated at the same level of information integration or could even link different levels of information processing. The important point of this hypothesis is the transient and dynamic nature of this link. Areas involved in the same functional processes could join a given network and synchronize their activities. Then, they could disengage and join a new assembly. Although attractive, this hypothesis has received so far only a few experimental supports. I will present some experiments we designed to address this point in the context of olfactory memory. We previously reported that performance improvement in an olfactory discrimination task is associated with the emergence in population recordings of oscillatory activities in the beta range (between 15 and 40 Hz) in the olfactory bulb and piriform cortex. These observations were recently extended to a larger network of structures using the olfactory aversion conditioning paradigm. Beyond phenomenology, the functional role of such oscillatory activities in learning and memory still remains open to discussion. We propose that mapping oscillatory activities in a network could be at least a good way to track learning-induced plasticity. We will also try to illustrate by some results how they could be used by structures in a network to dynamically interact as proposed by Varela.

Poster session I Poster #377

The feline taste for sour

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The taste world of the feline is distinctly different from that of most other mammals, in the pseudogenization of the Tas1R2 component of the sweet receptor. Among the remaining taste qualities, sourness is of particular interest within the petfood industry, where a common perception has been that cats prefer sour taste. pH is an important consideration for its impact on function and flavor, but data are lacking to support the perception that "sourness", per se, is a desirable taste quality for cats. Both astringency and sour taste are related to pH, but sourness is more directly correlated with titratable acidity, as well as other chemical attributes such as lipophilicity and molecular size. These sensory qualities are detected by distinct biological mechanisms. The purpose of this study is to develop methods for comparing intake of solutions by cats, to examine the sensitivity of cats to pH and to determine whether preference among various acidic solutions may be more directly related to pH, titratable acidity (sourness), or other chemical characteristics. Methods were approved by our Institutional Animal Care and Use Committee and included 4-bowl comparisons with group housed domestic cats for screening among multiple solutions and two bottle tests using 12 cats tested individually and trained to consume from standard licking spouts similar to those used in rodent studies. Behavioral observations were made at specified intervals to characterize the ingestive and non-ingestive bowl- or bottle- oriented responses such as sniffing and rubbing. Results indicate that: 1) there is a steep acceptance-avoidance curve for water solutions adjusted with HCl to pH's ranging from 3 to 7; 2) only a subset of cats preferred the more 'sour' of two solutions of equal pH that varied in titratable acidity; 3) cats appeared to use both smell and taste to determine preference for citric acid solutions, although these solutions could not be discriminated by humans by odor alone.

Poster session II Poster #86

Smelling the difference: chemical and sensory correlates tohost preferences in ovipositing insects

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The reproductive success of herbivorous insects depends on the mother's egg laying preference. In nocturnal insects, olfaction is the most important sensory modality mediating host location. Most gravid females select among a number of plants of varying suitability, yet assessments of the neuroethological mechanisms underlying odor-guided host choice are rare. In the hawk moth, *Manduca sexta,* a model for olfactory research, we show that gravid females perform a hierarchical choice among plants of different species and qualities using olfactory cues. Volatile profiles from these plants are clearly distinguishable between both plant species and qualities, and olfactory sensilla on female antennae detect more than half of the about 120 analytically detected volatiles in headspace samples. This reduced set of physiologically active compounds allows discrimination of species and quality. Olfactory sensory neuron assemblies present in antennal sensilla are mainly broadly tuned to many compounds, but some assemblies exhibit species and condition-specific responses. We suggest that the odor image, a sensory fingerprint of a host, is already represented at the sensory periphery, distinguishing host species and quality. We also show that a complex synthetic volatile blend is required to elicit attraction in egg laying females, while few compounds successfully mimic a flower to nectar-foraging females.

Our findings were obtained in a specific context: While a herbivore mother strives to improve her reproductive success, the host plant benefits from hiding. In contrast, clearly discernable scents signal the presence of nectar rewards to pollinators. Similarly, plants signal to parasitoid wasps not only the presence, but even the most appropriate state of their herbivorous prey. Findings in these different systems will be reviewed and compared to our results to discuss common principles of olfactory host choice but also divergent selective pressures under evolutionary perspectives.

Poster session I Poster #333

Modulating TRPM5 activity alters Benzamil (Bz)-insensitive NaCl Chorda Tympani (CT) Taste Nerve Responses

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We investigated if the Bz-insensitive NaCl CT response is altered by modulating TRPM5 activity in taste cells by monitoring CT responses to 100 mM NaCl+5 μM Bz in Sprague-Dawley rats, wildtype (WT) mice, TRPV1 knockout (KO) mice, TRPM5 KO mice and PLCβ_2 KO mice in presence of resiniferatoxin (RTX; 0-10 μM), a TRPV1t agonist. Rat CT responses to NaCl+Bz+RTX were also monitored after treating the tongue with triphenylphosphine oxide (TPPO), a TRPM5 blocker or capsazepine (CZP), a TRPV1t blocker. In rats, WT mice and PLCβ_2 KO mice, RTX produced a biphasic effect on the NaCl+Bz CT response with a maximum increase in the response around 1 μM. In rats and WT mice, the CT response to NaCl+Bz+SB-366791 (1 μM) or in TRPV1 KO mice the CT response to NaCl+Bz was inhibited to the rinse baseline level and was unaffected by RTX. Stimulating the rat tongue with NaCl+Bz+CZP (10 μM) or after treating the tongue with 2 mM TPPO inhibited the tonic CT response to NaCl+Bz and produced a rightward shift in RTX concentration versus the magnitude of the tonic NaCl+Bz CT response relationship. TRPM5 KO mice demonstrated no CT response to NaCl+Bz above rinse baseline and a rightward shift in the RTX concentration versus the magnitude of the tonic NaCl+Bz CT response relationship. Following topical lingual application of TPPO, adding 5 mM ATP to the NaCl+Bz+RTX stimulating solutions enhanced the magnitude of the rat CT response and shifted the RTX concentration versus the magnitude of the tonic NaCl+Bz CT response relationship to the left. We conclude that inhibiting the TRPM5 channel in fungiform taste bud cells attenuates the Bz-insensitive NaCl CT response and the sensitivity of TRPV1t to its agonists.

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Poster session II Poster #220

Role of the noradrenergic system in olfactory learning and adult neurogenesis.

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The main olfactory bulb is the first cortical relay involved in the transmission, processing and integration of olfactory information. It is also one of the brain regions receiving strong noradrenergic innervation from the *locus coeruleus*. Noradrenergic inputs play a critical role in several olfactory functions, such as odor discrimination, and various forms of olfactory memory. In addition, the olfactory bulb is one of the rare brain regions where neurogenesis persists during adulthood. Adult neurogenesis is differently required for specific forms of olfactory learning. Indeed, neurogenesis is necessary for the acquisition of perceptual learning (a form of learning where two undiscriminated odors become discriminated after a 10-day exposure to these two odors), but only for the long term retention of associative olfactory memory (the animal learns to associate a reward to the presence of a specific odor).

In this study, we aimed at identifying the cellular and molecular basis of the different requirement of neurogenesis in perceptual and associative olfactory learning. We first investigated the role of noradrenaline in these two forms of learning. We performed *in vivo* intrabulbar infusion of noradrenergic receptor antagonist to locally block the noradrenergic signaling pathway during perceptual and associative learning. We showed that bulbar noradrenaline is necessary for perceptual learning but not for associative olfactory memory.

We then investigated the mechanisms linking adult neurogenesis, olfactory learning and noradrenaline. We showed that newborn neurons receive noradrenergic synaptic contacts at crucial time points during their maturation and integration in the bulbar neuronal network.

Altogether our results support the hypothesis of noradrenaline being a key regulator of adult neurogenesis during olfactory learning.

Symposium 5 "Interspecific chemointeractions" Sunday 24 June

Tracking a dynamic odor plume in a noisy chemical environment

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Natural, behaviorally significant olfactory stimuli typically are mixtures of volatiles whose concentrations co-vary dynamically in time and space. The olfactory world is also an arena of constant movement and flux. Once emitted by a source, volatiles are dispersed and mixed by the ambient motion of air to form a shifting and filamentous plume. How does the olfactory system quickly discriminate the fluctuating signal from a background? We examined this process using the moth, *Manduca sexta*, and the volatiles from its hostplant, *Datura wrightii*. Using proton-transfer-reaction mass spectrometry, the fluctuating odor plume from *D. wrightii* flowers were characterized in field sites near Tucson AZ USA. Results from these measurements demonstrated that, even within the headspace of the flower, the ion plume was highly dynamic in both time and space. Moreover, with increasing distance from the source, the chemical background became mixed with the flower plume. We examined the ability of the moths to encode the temporally dynamic signal by multi-channel recording in the moth's antennal (olfactory) lobe (AL). The neural ensemble could track the mixture of the flower odor at temporal frequencies up to 5 Hz. However, when an odor background was presented, the ensemble representation of the flower mixture decreased. The change in AL representation was due to the changing of the ratios in the presented stimulus, thereby altering the balance of excitation and inhibition in the system. Finally, behavioral experiments in a wind tunnel showed that the ability to track a plume significantly decreased when background odors of increasing similarity to the floral mixtures were presented. Together, these results provide new evidence that in moths, upwind orientation to mixtures is mediated by the precise integration of multiple glomerular pathways, and that alteration of the mixture input transforms the network representations.

Poster session II Poster #60

Brain-behavioural lateralization in honeybees: odour dependent asymmetry and a morphological comparison of the Antennal Lobes

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Recently, a lateralization in the recall of odour memories was revealed in the honeybee, *Apis mellifera,* showing a right dominance on short-term odour retention. In addition, it was demonstrated that odour detection in honeybees is not equal between the antennae, with the right one showing higher level of depolarization after odour presentations and higher number of olfactory sensilla. Here we want to present results on the anatomical measurements between the right and left side in the first olfactory centers of the honeybee brain, the Antennal Lobes (ALs). A subset of the ALs' functional units, the glomeruli, was imaged using two-photon microscopy, and volumetrical reconstructions were compared between sides. Furthermore, we performed single-antenna recall test conditioning bees to extend their proboscis (in the so-called PER paradigm) in association to those odours that more strongly activated functional responses in the selected glomerular subset. Anatomical analysis did not reveal significant differences between sides but the behavioural tests showed an odour dependence in the capacity of bees to recall compounds with the two antennae. These data provide new evidence on the odour effect on behavioural asymmetries in honeybees as well as on the quest for the anatomical differences beyond the lateralized behaviour.

Poster session I Poster #215

Modulation of olfactory epithelium cellular dynamics by endothelin in vivo

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Based on its accessibility and its plasticity, the olfactory mucosa is a good model to understand the mechanisms underlying the regeneration of nervous tissue. Olfactory mucosa expresses many factors modulating neuronal survival. Among them we had previously shown that endothelin plays an anti-apoptotic role *in vitro*. In order to elucidate its role *in vivo*, we have treated newborn rats with endothelin receptors antagonists, applied by intranasal instillations twice a day. After one week of treatment, the olfactory epithelium from treated animals displayed increased apoptotic signal (TUNEL) in all cell layers and decreased amplitude of response to odorants measured by electro-olfactogramm (EOG). Those changes are consistent with an antiapoptotic role of endothelin in the olfactory epithelium *in vivo*. Despite the decrease of EOG amplitude signals, treated animals performed better in an olfactory orientation test. As the orientation behavior test was based on maternal odor recognition, the treatment may improve the animal performance toward an odorant present in its environment.

In order to test this hypothesis, we chose to follow a specific population of olfactory sensory neurons sensitive to octanal (expressing the rat I7 olfactory receptor). In the pro-apoptotic context induced by the treatment with endothelin receptor antagonists, octanal exposure induced an increase in I7 transcript level in a dose-dependant manner. These results suggest a close interaction between neurotrophic factors and activity-dependant survival of olfactory neurons.

Poster session I Poster #135

Classification of excitatory neurons in layer III of the mouse piriform cortex

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With its relatively simple trilaminar structure, the primary olfactory (or piriform) cortex is an appealing model for studying cortical sensory processing. The piriform cortex (PC) is also unusually susceptible to epilepsy. Despite much work showing the importance of neurons in layer III of the PC for seizure initiation and propagation, no studies have rigorously classified layer III excitatory neurons. Our aims were to quantitatively classify glutamatergic neurons in layer III of the PC and to explore their possible roles in epileptogenesis. **Methods:** Experiments used acute slices from 18-25 d-old GAD67-GFP mice in which GABAergic neurons express GFP. GFP-negative neurons were selected for whole-cell patch clamping and electrical characterisation. Electrodes contained 0.4% biocytin for recovery of morphology. **Results:** The dataset comprised >40 neurons distributed across layer III. Unsupervised cluster analysis of morphological parameters identified two major classes, pyramidal cells and multipolar cells. These classes differed significantly in several electrical properties, including input resistance and kinetics of their excitatory postsynaptic currents. Both cell types received strong epileptiform excitation when GABAergic synaptic inhibition was blocked with picrotoxin. **Conclusions:** Layer III of the PC contains two morphologically distinctive classes of glutamatergic neurons that differ subtly in their electrical properties. Both appear to be equally engaged in seizure activity. Future work will examine in more detail the roles of layer III pyramidal and multipolar cells in the function and dysfunction of this olfactory circuit.

Poster session I Poster #305

Assessing olfactory learning and memory in the 5xFAD mouse model of Alzheimer's disease.

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As a result of continued advances in genetic engineering techniques, an ever-increasing number of mouse models of Alzheimer's disease (AD) are being developed. Before these animals can be used to assess the potential of novel drug therapies, the validity of the models must be tested. Despite the well-known capacity of rodents for learning olfactory tasks, the majority of memory tests rely upon visual cues. In our lab, we are using an operant-olfactometer to assess olfactory working memory in male and female mice of different ages. Thus far, we have assessed learning in 6 month old males and females of the 5xFAD mouse model of AD. The mice successfully completed an olfactory discrimination task. The 5xFAD animals took longer to complete a reversal learning test compared with wildtype littermates. Both groups were able to complete a delayed matching to sample task with delays up to 30 seconds. These results suggest that the pathology associated with the 5xFAD model produces perseveration deficits in animals in this age group.

Symposium 1 "The other noses – the vomeronasal organ, the septal organ and the Grüneberg ganglion" Saturday 23 June

Emergence of novel chemosensors: from the immune to the olfactory system

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In mammals, natural selection has generated various olfactory chemosensory organs to face the outside world. These structures use diverse molecular receptors that are characteristic of each species. The origin of this diversity is multiple: it often results from a preexisting olfactory gene repertoire, that is amplified and susequently mutated. But it can also emerge from the hijacking of receptor genes initially not expressed in the olfactory system. Thus, formyl peptide receptor (FPR) genes, that are found in all mammals, are expressed in immune cells in which they recognize disease and pathogen-related molecules. Recently, in some rodent species, gene duplication events followed by gene cluster invasion led to the intermingling of vomeronasal receptor and FPR genes. This accident correlates with a drastically different expression pattern of most FPRs in these species: their transcription is absent from immune cells, and is restricted to sensory neurons of the vomeronasal organ. The peculiar agonist profile of these vomeronasal FPR receptors, and their maintenance in multiple rodent species, suggest that this acquisition may be of selective advantage. Taken together, this rodent-specific chemosensory tool may represent a clean example of evolutionary novelty, resulting from genomic landscape alterations that led to the hijacking by FPR genes of cis regulatory sequences from neighbouring genes.

Symposium 8 "Central mechanisms of taste learning and memory" Sunday 24 June

Cognitive and attentional modulation of taste processing in the brain

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Recordings of neuronal activity, and functional neuroimaging in humans, show that the primary taste cortex in the anterior insula provides separate and combined representations of the taste, temperature, and texture (including fat texture) of food in the mouth independently of hunger and thus of reward value and pleasantness. One synapse on, in the orbitofrontal cortex, these sensory inputs are for some neurons combined by associative learning with olfactory and visual inputs, and this type of learning can be reversed rapidly (in one trial) for visual-taste associations, and more slowly (in 40 trials) for olfactory-taste associations. By olfactory-taste associations, a representation of the flavour of food is formed. These neurons encode food reward in that they only respond to food when hungry, and in that activations correlate with subjective pleasantness. The mechanism for sensory-specific satiety involves synaptic adaptation over a number of minutes far on in taste and olfactory processing, in the orbitofrontal cortex, but not at earlier stages of taste processing.

Cognitive factors, including word-level descriptions, and attention, modulate the representation of the taste and olfactory reward value of food in the orbitofrontal cortex. Selective attention to the pleasantness of a taste vs its intensity modulates activations in the orbitofrontal and pregenual cingulate taste cortex vs activations in the insular (primary) taste cortex.

After the reward value of stimuli has been represented in the brain, a choice decision is taken by a third cortical tier of processing in the ventromedial prefrontal cortex, area 10, and this decision-making is probabilistic because of the randomness of the firing times of populations of neurons.

Rolls, E. T. (2011) Chemosensory learning in the cortex. Frontiers in Systems Neuroscience 5: 78.

Symposium 19 "Preference for umami taste controlled by chemical senses - Ajinomoto Symposium" Tuesday 26 June

What makes umami pleasant? Multimodal convergence, and top-down attention and cognition

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The cortical processing of umami reveals that the pleasantness of umami reflects and is correlated with processing in the secondary taste cortex in the orbitofrontal cortex and tertiary taste cortex in the anterior cingulate cortex, whereas processing in the primary (insular) taste cortex reflects physical properties such as intensity.

However, glutamate (an umami taste stimulus) presented alone as a taste stimulus is not highly pleasant, and does not act synergistically with other tastes (sweet, salt, bitter and sour). When glutamate is given in combination with a consonant, savory odor (vegetable), the resulting flavor, formed by a convergence of the taste and olfactory pathways in the orbitofrontal cortex, can be much more pleasant, and this is reflected in supralinear activations in the orbitofrontal cortex. Glutamate can thus act to enhance the pleasantness of food by combining supra-linearly with consonant odors in cortical areas where the taste and olfactory pathways converge far beyond the receptors.

Top-down cognitive effects onto the orbitofrontal and pregenual cingulate cortex also interact with the taste and favour of umami to heighten the palatability of umami. Further, top-down selective attention to pleasantness enhances the responses in the orbitofrontal and pregenual cortex processing stream. Attention to intensity enhances the responses in the primary taste (insular) cortex. Attention-based biased activation of affective vs identity processing cortical streams is thus an important principle of cortical processing related to how food stimuli including umami are processed in the brain.

Rolls, E.T. (2009) Functional neuroimaging of umami taste: what makes umami pleasant. American Journal of Clinical Nutrition 90: 803S-814S. Grabenhorst, F. and Rolls, E.T. (2010) Attentional modulation of affective vs sensory processing. J Neurophysiology 104:1649-1660. Papers at http://www.oxcns.org

Poster session I Poster #165

Response to all or parts of the whole: a case of configural perception in the newborn rabbit

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Coming from flowers, foods or conspecifics, odours are ubiquitous in our environment. Most of the time, perceived odours are the result of complex relationships between tens or more of odorants with our olfactory system. Two perceptual strategies are potentially engaged to perceive such chemically complex mixtures. The elemental mode allows the perception of each component's quality. Conversely, the configural mode supports the perception of a new odour in addition to, or in place of, the odours of the components (blending effect). In humans, it is usually considered that the prevalence of one mode on the other is due, in part, to mixture complexity. In the rabbit, previous results have shown that newborns perceive in a configural mode a senary mixture, which gives rise to a blending effect in human adults (mixture smelling like red cordial; RC). Indeed, after learning of one component, newborn rabbits do not respond to the RC mixture. In the same time, they perfectly respond to a mixture including the same chemical components but at a different ratio, or to another senary mixture not blending in humans. Here, we tested the hypothesis that learning a sufficient number of components may induce responsiveness to the complex RC mixture initially processed following the configural mode. To that goal, we compared the pups' responsiveness to RC after learning of one, two, three or more of the components with the aim to assess whether the components' perception is not at all possible in RC (robust configuration), or if the generalisation is possible depending on the number of components learned (weak configuration). Results suggest a weak configural perception of RC, and a behavioural responsiveness dependent on the knowledge the newborns have of the components.

Poster session II Poster #408

Pannexin 1 does not form ATP-permeable channels in a heterologous system

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Mammalian taste cells of the type II release the afferent neurotransmitter ATP through ATP-permeable channels. Panx1 hemichannels are considered as most likely candidates for such channels, although evidence suggests that connexons can not be excluded. Here we carried out the comparative analysis of biophysical and pharmacological features of recombinant Panx1 and ATP-permeable channels operative in type II cells. Panx1 was cloned from the taste tissue and heterologously expressed in eukaryotic cells of several lines, including HEK-293, CHO, and neuroblastoma SK-N-SH. It was shown that the heterologous expression of Panx1 in cells of either type originated outward rectifier currents effectively blockable with carbenoxolone, probenecid, and anionic channel blockers, such as DIDS and NPPB. In transfected but not control cells, Panx1-dependent carbenoxolone-sensitive currents were detectable even at high negative voltages. Ion substitution experiments pointed to basically anion selectivity of Panx1 hemichannels, which were poorly permeable to anions larger than 200 Da. Apparently, ATP was a blocker rather than a permeant species for Panx1 hemichannels. In excised patches, transfection of HEK-293 cells with Panx1 cDNA gave rise to single channel-like current events suppressed by carbenoxolone and probenecid. The individual carbenoxolone-sensitive channels exhibited outward rectification. Although the open-channel probability strongly increased with membrane voltage, Panx1 channels were active at high negative voltage. Unlike taste cells, ATP release from Panx1-positive HEK-293 cells was undetectable. Thus, biophysical and pharmacological features of recombinant Panx1 hemichannels suggests that either they do not serve as the main conduit of ATP release or in taste cells, ATP-permeable channels are heterooligomeric complexes of Panx1 and other channel subunits.

Symposium 12 "No taste, no smell: When the chemical senses are lost " Sunday 24 June

Parosmia and Phantosmia into the clinic for patients with olfactory disorders

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Chemosensory dysfunction is usually categorized into quantitative and qualitative disorders. Qualitative disorders are represented by **parosmia** (distorted perception of odors in the presence of an odor source) and **phantosmia** (perception of an odor in the absence of an odor source). Presence of parosmia has been evaluated at 34% of the patients complaining of an olfactory dysfunction with a different prevalence depending on the etiology of the olfactory trouble; 56% for post-infectious olfactory loss, 10% in idiopathic, 14% in post-traumatic, and 28% in sinonasal related olfactory dysfunction. Phantosmia is less frequently described with 12% of all patients. Parosmia may have a negative or a positive hedonic valence for the patient and is often perceived as more annoying than a quantitative disorder. Depending on the state of the disease presence of parosmia may be a sign of recovery of olfactory function; whereas phantosmia tendentially predicts decrease of olfactory function.

Basic physiopathologic mechanisms, prevalence in our Taste and Smell clinic, standard evaluation, treatment and most approved studies on this topic will be discussed in this lecture.

Contributed talks V "Human olfaction" Monday 25 June

Mental imaging in professional perfumers, cooks and musicians: linguistic and EEG studies

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There are large individual differences in the ability to form vivid mental images. Experience accounts for some of this variability and its long-term effect can be seen in creative thinkers, such as chefs, perfumers or musicians. We interviewed 33 creators in perfumery, gastronomy and music to assess how they represent virtual smells or virtual sounds and act on them. The methodology was both qualitative and quantitative: linguistic analysis aided by the textometry software HyperBase. Results showed that like musicians who hear music "with the mind's ear", perfumers and cooks also have the ability to "smell in their mind". These olfactory images usually concur with visual representations and creativity often involves conceptual blending between vision and other modalities. In an EEG study we tested olfactory and auditory imagery ability in 13 cooks, 15 musicians and 15 lay controls to assess whether mental imaging ability of professionals is reflected in their brain activity. Evoked potentials were recorded using a 64-channels EEG system during imaging of the odor of fruits, and of the timbre of music instruments. Control visual tasks consisted in imaging the size of fruits and instruments.

At the behavioural level, whereas cooks responded faster during odor imagery than size imagery for fruits, musicians responded faster during timbre imagery than size imagery for instruments. Controls did not differ between these tasks. At the neural level, a hemispheric dissociation emerged for a late positive complex (time window: 800-1200ms): (1) tasks involving mental imagery of size (fruits or instruments) involved significantly more strongly the left hemisphere in all three groups, (2) odor imaging recruited equally strongly both hemispheres in cooks, (3) timbre imaging recruited equally strongly both hemispheres in musicians. These findings suggest that sensory experience of creative thinkers involves a domain-specific brain modulation of mental imaging ability.

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Poster session II Poster #346

Pigs show no preference for low concentrations of polycose, maltose and several polyols and a high preference for ethanol.

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It has been proposed that there are two different sets of carbohydrate taste receptors in mammals: the well-known sweet receptor (the dimer T1R2/T1R3) and a polysaccharide receptor. The sweet receptor seems to be involved in signalling triggered by sugars and Ethanol while the second receptor would signal complex carbohydrates such as Polycose (a commercial Maltodextrin). In rats and mice Polycose and maltose at low concentrations are preferred over sugar. Preliminary double choice tests in pigs showed that the sugar preference threshold was around 10 mM. However, the preferences for Maltodextrins and Ethanol in pigs are not known. The present pig study used a 2 minute double choice model based on simultaneously offering two dishes containing either water or a test solution. The solutions tested were sugar (at 200 mM), a positive reference, or: Polycose (at 0.1, 0.5, 1.0 and 1.5 %), Maltodextrin (at 0.1, 0.5, 1.0 and 2.0%), Dextrose (at 10, 20, 30 and 40 mM), Maltose (at 3, 15, 30 and 60 mM) and Ethanol, Xylitol, Erythritol, and Maltitol (at 5, 10, 15 and 20 mM). Each solution was tested in eleven pairs of piglets of 5 weeks of age. Potential positional effects of dishes were voided by alternating the left and right positions of the dishes in each pen (6 and 5 pens in each side). Preference values were compared to the neutral value of 50% and significance was set at a $P<0.05$. As expected, pigs chose sugar at 200 mM over water with a significant preference of 72%. None of the concentrations tested for Polycose, Maltodextrin, Maltose and the polyols Xylitol, Erythritol and Maltitol resulted in a significant preference. Pigs, in turn, showed a significant preference for Dextrose at 10 mM only. However, the highest significant preference of all the non-sugar solutions tested was the 67.8% reached with the 20mM Ethanol. It is concluded that the pig preferences for Ethanol but not Polycose and Maltodextrines advocates the pig as a human model for taste studies.

Poster session I Poster #347

Pigs show no preference for low concentrations of several cereal starches, potato starch and tapioca and a high preference for hydrolyzed corn starch.

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Starch-rich foods have a high nutritional value and are a fundamental part of the diet of most mammalian species. Previous data in 4-day double choice tests showed that pigs have high preferences for specific starch sources in complete feeds. The higher the glucose availability from the starch source the higher the preference (an significant –$P<0.05$- Pearson's correlation of $r=0.48$ was found between preference and "in vitro" glucose release). However, the 4-day tests may have been influenced by the interactions with other feed ingredients of the diet and the effects on the hunger satiety cycle. In contrast, the short-term model used in this study targets peripheral sensing, particularly taste. We used a 2 minute double choice pig model based on simultaneously offering two dishes containing either water or a test solution. Sugar (200 mM) was used as a positive reference. The solutions tested were: Tapioca (T) or Potato (P) starch (at 1, 2, 3 and 4%) or Wheat (W), Rice (R), Corn (C), Corn waxy (CW), Corn high amylose (CA) or hydrolysed corn (HC) starch (at 0.1, 0.5, 1.0 and 2.0%). Each solution was tested in eleven pairs of piglets (mean body weight 13.5 kg). Potential positional effects of dishes were voided by alternating the left and right positions of dishes in each pen (6 and 5 pens in each side). Preference values were compared to the neutral value of 50% and significance was set at a $P<0.05$. Sugar was significantly preferred over water with a 77% ratio. Preference for 0.1% solution of W starch (38.83%) was significantly lower than 50%. None of the other solutions of T, P, W, R, C, CW or CA starches tested resulted in a significant preference. Pigs, in turn, showed a significant preference of 65.83% for the 1% solution of HC starch. It is concluded that the pigs do not show a short-term preference for starches in water solution except when hydrolysed. The effect of technological treatments on starch preference in pigs should be further investigated.

Poster session I Poster #61

Can praying mantids taste bitter toxins?

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Praying mantids are ferocious predators. Research has predominantly focused on their visual perception, and how they use motion and contrast to detect and strike at prey. Consequently, we know very little about how these insects use taste in their foraging decisions. Of particular interest is how these predators might detect bitter-tasting toxins upon attack, either by using their forelimbs or their mouthparts. Being able to detect toxins in this way could prevent them ingesting harmful toxins, and allow prey to escape relatively unharmed. In a series of experiments, we investigated the bitter taste perception of two mantis species: the giant asian mantis (*Hierodula membranacea*) and the African lined mantis (*Sphodromantis lineola*). We find no evidence that they can detect bitter toxins on their forelegs, but show that they can detect them on their mouthparts with increasing concentration. However, there may be differences in sensitivity between the two species, and potentially also between the sexes. We discuss these findings in the context of the evolution of prey defences.

Contributed talks II "Gustation" Monday 25 June

Distastefulness as a signal of toxicity

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Toxic insects often advertise their defences to predators using conspicuous warning coloration. However, many toxins are also bitter-tasting and can be detected by a predator during an attack. This potentially allows prey to signal to predators how much toxin they contain, and for predators to taste and reject those prey they consider to be too toxic to eat. We present data from experiments with birds showing that they are able to use taste to detect differences among prey containing different concentrations of quinine and preferentially ingest the less defended individuals. By independently manipulating prey toxicity and distastefulness, we also show that birds learn to use bitter taste to discriminate between prey containing different amounts of toxin. However, birds only rely on taste information when distastefulness correlates with toxicity. This suggests that predators exert strong selection pressure on prey to reliably signal their toxicity using distasteful signals. We discuss these findings in relation to the evolution of defence strategies in toxic prey.

Poster session II Poster #62

Synaptic circuitry of identified neurons in the antennal lobe of *Drosophila melanogaster*

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Olfactory cues are processed in multiple microcircuits in the first olfactory relay station of the *Drosophila melanogaster* CNS, the antennal lobe (AL). The glomerular synaptic network is principally comprised of receptor neurons, local interneurons and projection neurons. In a correlative LM-EM analysis we are dissecting this circuitry by combining confocal microscopy, 3D models and reconstructions of serial electron microscopy (EM) sectioning.

Method AL output neurons (projection neurons, PNs) were labeled using a genetically encoded membrane-bound EM

marker: Horseradish peroxidase: HRP::CD2 (Watts et.al. 2004) driven by the Gal4-line GH146. This made possible to trace and reconstruct with high reliability synaptic sites in PNs. **3D model** In order to identify individual glomeruli at the EM level landmark structures were segmented from panoramic EM views of the complete AL. This 3D model was then registered to a template glomerular AL map (nomenclature: Laissue et al. 1999) derived from reconstruction of brains immunostained with the synaptic marker nc82. **Identification of PN synapses** We have found PN input and output synapses, with characteristic T-shaped densities, consisting of platform and pedestal, as well as synapses between PN profiles. The most common synaptic constellation was a tetrad, i.e. a presynaptic profile, opposed to four postsynaptic profiles containing postsynaptic densities. A conspicuous feature, found in yet unidentified, presumably local interneuron processes, is the presence of T-bars with an elongated platform and several pedestals forming multiple connection to up to 5-6 postsynaptic processes, including PN profiles. **Outlook** Currently, we are extending our analysis of AL synaptic circuitry to include receptor neurons and local interneurons.

Laissue et.al (1999) Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*. J comp Neurol405(4).
Watts et.al (2004) Glia engulf degenerating axons during developmental axon pruning. Curr Biol 14(8)

Symposium 18 "Olfactory neuroethology" Tuesday 26 June

Structure and function of olfactory circuits in *Drosophila*

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We are investigating how odors are coded and processed in the *Drosophila* brain to lead to a specific odor perception. The basic layout of the first olfactory processing centers, the olfactory bulb in vertebrates and the antennal lobe (AL) in insects, is remarkably similar. Odors are encoded by specific ensembles of activated glomeruli (the structural and functional units of the bulb-lobe) in a combinatorial manner. The vinegar fly *Drosophila melanogaster* provides an attractive model organism for studying olfaction, as it allows genetic, molecular and physiological analyses. We are performing calcium as well as chloride imaging of ecologically relevant odors to decipher basic principles involved in coding, processing and perception of odors. We will present our recent insights into olfactory coding strategies yielded by morphological and functional analysis of the different neuronal populations present in the *Drosophila* antennal lobe and aim to find the link to odor- guided behavior.

Poster session I Poster #269

Odors, a privileged way to access memories. A novel behavioral approach of episodic memory in human.

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Odors are known to be especially evocative reminders. The odor-evoked memories are more emotional, more associated with subjective experience, and more vivid than those recalled by other sensorial cues. This really strong connection between olfaction, emotion and memory make olfaction a privileged sense to access to memories. The main goal of our study is to create a novel approach to investigate episodic memories triggered by odors.

In our approach, the to-be remembered episodes were rich and as close as possible to real-life situation. They were made up of three dimensions: three odors (*what*), positioned on specific spots of a board (*where*) and presented in a visual context, the picture of a landscape (*which context*). Odors were chosen to be highly difficult to identify in order to favor perceptual features and to limit the use of verbal label. During encoding, subjects freely discovered episodes, one episode a day, over three consecutive days. On the fourth day, episodic memory triggered by odor was explored. When an odor was recognized as being part of a previous episode, the subject was asked to remember both its spatial location and the visual context in which it occurred.

Behavioral data showed that subjects were highly competent at recognizing unfamiliar odors smelled during encoding. When a target odor was recognized, subjects were able to recall the spatio-contextual environment of episodes in about half of the trials. Trials repetition is needed for the use of this paradigm in an fMRI study in order to increase the signal quantity and subsequently to improve its quality. Results showed that multiple presentation of a same odor for recognition and episodic recall did not disturb memory process.

In brief, the current study first validates our novel paradigm designed for episodic memory study, and second demonstrates its adaptability to fMRI protocol in order to explore neural networks implicated in episodic recall.

Poster session I Poster #349

Trigeminal sensation of the tongue after middle ear surgery

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OBJECTIVE: After middle ear surgery, patients often complain of tongue numbness and taste disorder. The mechanism of trigeminal sensations such as numbness is unclear; therefore, we assessed changes in trigeminal sensation of the tongue in middle ear surgery patients.

SUBJECTS: 91 patients (49 males, 42 females; aged 7 to 70 y [mean, 37.7 y]) underwent surgery from October 2009 to October 2011. They had unilateral ear diseases, and had not undergone previous ear surgeries. In 23/91 patients, the chorda tympani nerve (CTN) was sectioned during surgery. In 68/91 patients, the CTN was preserved.

METHODS: In all cases, Semmes-Weinstein sensory test (SW test), 2-point discrimination test, and electrostimulator test (STG 4002®, Multi channel systems, Germany) were performed before surgery and 14 days after surgery. Taste function was assessed with electrogustometry (EGM) (TR-06®, RION Co., Japan). These 3 tests and EGM were performed at the ridge 2 cm behind the tip of the tongue on each side (the region controlled by the CTN).

RESULTS: Tongue numbness occurred in 15/23 (69.6%) patients with CTN sectioning and in 18/68 (27.9%) patients with preserved CTN ($p<0.01$). Taste disorder occurred in 14/23 (65.2%) and 21/68 (33.5%) patients, respectively ($p<0.05$). In patients with CTN sectioning, postoperative thresholds on the operated side were significantly higher than preoperative thresholds for the electrostimulator test and 2-point discrimination test ($p<0.05$ for both). In patients with CTN preservation, postoperative thresholds were significantly higher than preoperative thresholds on only the electrostimulator test ($p<0.01$). In 4 patients who underwent inlay myringoplasty, the CTN was manipulated during surgery; however, no differences between preoperative and postoperative thresholds in the 3 tests and EGM were found.

CONCLUSIONS: These findings suggest that damage to the CTN during middle ear surgery induces trigeminal sensations together with taste disorder.

Poster session I Poster #271

Processing of mint flavor activates different brain areas from those of mint aroma.

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Minty flavors are utilized in foods, beverages, toothpastes and tobacco. Minty flavors are considered to have a relaxing effect, which is necessary for us, living in modern and urban societies. The authors have reported that the participants evaluated themselves more relaxing after smelling minty odor, when they were tied out for solving hard puzzles.

However, psychological and physiological effects of minty flavors are still unclear. This study was aimed to reveal brain responses to minty flavors and minty odors with NIRS (near infrared spectroscopy) and functional MRI.

In NIRS experiment, 60 female university students participated. They received detailed explanation about the experiment and wrote consent for participating in this study. The three minty stimuli, menthol, spearmint and peppermint and the other three odor stimuli, rose, skatol and orange, were administered to the participants via olfactometer. The participants were asked to smell the stimuli by orthonasal route, retronasal route or to taste them. Brain responses were measured with NIRS (BOM- 1, Omegawave, CO LTD) and recorded with AD converter (Powerlab) connected to the iMac. The participants were also asked to evaluate the intensity of the odor continuously with the apparatus which outputs 0~5 V being based on the participants' evaluations.

The results showed that the stimuli were recognized faster and stronger when the stimuli were administrated via orthonasal and retronasal route than via oral cavity. The rose odor and orange odor were recognized faster and stronger than the menthol odor. The right forebrain showed greater response to the stimuli via orthonasal route than via the other routes, and greater response to the rose odor than the menthol and the peppermint odors.

In fMRI experiment, 10 volunteers participated. The same odorants and same stimulation routes described above were adopted in this experiment. The results showed the brain areas activated by each stimulus varied.

Symposium 10 "From odorant receptor to glomerulus" Sunday 24 June

Neural map and circuit formation in the mouse olfactory system

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In the mouse olfactory system, much of axon wiring in neural map formation occurs autonomously by axon-axon interactions of olfactory sensory neurons (OSNs). Axonal projection along the dorsal- ventral (D-V) axis is regulated by positional information of OSNs within the olfactory epithelium (OE). Axon guidance molecules, such as Nrp2 and Sema3F expressed in OSNs, determine D-V positioning of glomeruli. Unlike the projection along the D-V axis, anterior-posterior (A-P) projection is instructed by OR molecules that regulate the expression levels of axon guidance molecules using cAMP as a second messenger. How is it, then, that cAMP signal levels are uniquely determined by each OR species? Many G-protein coupled receptors (GPCRs) are known to posess two different conformations, active and inactive, and spontaneously transit between the two, generating the basal activity in the absence of agonists. We assume that the OR-derived basal activity participates in the olfactory map formation. Beta2-adrenergic receptor (beta2-AR) is known to share many functional similarities with OR molecules and substitute them for OR-instructed OSN projection. Taking advantage of these previous observations, we have analyzed axonal projection of OSNs instructed by the mutant-type beta2-AR with the altered levels of basal activity. We have found that the basal activity mutants alter expression levels of axon guidance molecules, e.g., Nrp1 and PlxnA1, and change glomerular locations along the A-P axis. After establishing the topographic map, it needs to be properly connected with mitral/tufted (M/T) cells. We have found that Sema3F, a repulsive ligand to Nrp2, which is secreted by early-arriving dorsal OSN axons, guides both late- arriving $Nrp2^{+}$ OSN axons and $Nrp2^{+}$ M/T cells to the ventral region of the OB. This coordinated guidance is an important process for proper alignment and synapse formation of OSN axons and M/T cells during development.

Poster session I Poster #221

Analysis of the mouse olfactory transcriptome by deep RNA sequencing

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There is increasing evidence that the main olfactory and the vomeronasal systems have overlapping functional roles in mediating chemosensory perception, yet little is known about how this is achieved at the molecular level. We characterized the transcriptome of both the vomeronasal organ (VNO) and the main olfactory epithelium (MOE) by sequencing RNA on the Illumina platform, comparing global gene expression between three female and male mice. We collected 65-85 million reads per mouse per tissue, of which ~80% were successfully mapped to the reference mouse genome, and calculated the relative expression of over 36,000 unique transcripts, including all known chemosensory receptors. The accuracy of the technique was assessed using qRT-PCR, revealing a highly significant correlation in gene expression values between methods.

In the VNO, the primary pheromone detecting organ, the most abundant non-housekeeping transcripts encode secreted proteins including multiple lipocalins and the extracellular proteinase inhibitor, *Expi*. We also identified some highly expressed but entirely novel transcripts, reflecting our limited knowledge of genes that pattern the VNO. Less than 1% of the transcripts display a statistically significant difference (>1.5 fold) in abundance between males and females, indicating some sexual dimorphism in chemosensory detection or transduction. Vomeronasal receptors (VRs) are unequally expressed across a surprisingly large dynamic range; these abundances are very consistent between individual mice suggesting a non-stochastic VR selection in sensory neurons. Unexpectedly, we identified three olfactory receptors (ORs) expressed in the VNO in a consistent and specific manner, revealing a potential molecular mechanism for VNO-mediated odorant detection.

Using these data, with corresponding analysis of the MOE, we have now knocked out a number of candidate genes in mouse models to further understand the molecular basis of olfaction.

Poster session II Poster #378

Human TAS2R38 taste receptor genotypes have a role in consumption of lingonberry (*Vaccinium vitis-idaea*)

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Lingonberries (Vaccinium vitis-idaea) is the highest-yielding wild berry in the Nordic countries and has high content of bioactive compounds such as resveratrol, proanthocyanidins, flavonols, and vitamin E. It is either liked or disliked due to its unique orosensory properties and especially its hypothetical dominating sourness. We have previously shown that orosensory profile of natural lingonberry juice or puree is a mixture of astringency, bitterness, and sourness (1). These properties may have significant impacts on liking of natural berry. Because of the bitter taste we decided to explore the influence of hTAS2R38 taste receptor gene on self-reported consumption of lingonberry in Finland. All subjects were genotyped at three variable locations: AA 49, 262, 296 (2). A total of 107 adult subjects (22 - 66 years) were recruited and 89 of them (16 PAV/PAV homozygotes, 32 AVI/AVI homozygotes, 35 PAV/AVI heterozygotes, 6 AVI/AAV) completed an electronic food frequency questionnaire. Frequency was measured with a 5-point category scale (seldom or never, maximum twice per month, once per week, few times per week, daily). Non-parametric Kruskal-Wallis together with Mann-Whitney U tests were applied to data processing. The difference between the genotype groups was significant ($Chi2 = 8{,}619$, $p < 0.05$) in frequency of lingonberry consumption and PAV homozygotes used it less frequently than AVI homozygotes ($p < 0.01$). Moreover, 19 % of PAV homozygotes, but 54 % of AVI homozygotes reported to consume lingonberry at least once per week. In turn, only 14 % of AVI homozygotes but 44 % of PAV homozygotes consumed it less than once per month or never. The key bitter compounds of lingonberry are still unknown, but this study indicates that bitter taste and TAS2R38 taste receptor may have a role both in liking and consumption of lingonberry. This work was supported by Academy of Finland (MS252005, MS256176).

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Poster session II Poster #306

Lack of diurnal variation of sweet recognition thresholds in over-weight and obese humans

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Leptin is a circulating hormone that regulates food intake, energy expenditure, and body weight mainly via activation of the hypothalamic functional leptin receptor (Ob-Rb). In peripheral taste organ, leptin specifically inhibits gustatory responses to sweet substances without affecting responses to sour, salty and bitter substances in lean, but not diabetic mice. In humans, sweet taste recognition thresholds showed diurnal variation that parallel with variation of plasma leptin levels. In the present study, we examined potential linkages among leptin, glucose, insulin levels and sweet taste by measuring and comparing recognition thresholds for various taste stimuli, plasma leptin, insulin and blood glucose levels at 7 time points during the day in Japanese over-weight and obese (OW/Ob) adults (BMI > 25 kg/m^2). Plasma leptin and insulin were measured by an enzyme-linked immunosorbent assay. Blood glucose levels were determined by the glucose dehydrogenase method. In OW/Ob subjects with higher leptin levels (~20 ng/ml), there were no significant diurnal variations of mean recognition thresholds for sweet stimuli (sugars and saccharin). Individual differences in diurnal variation of leptin and recognition thresholds for sweet stimuli were negatively correlated with ratios of postprandial blood glucose increase vs. insulin increase. In OW/Ob subjects, basal leptin levels may already be higher than leptin's effective range for modulation of sweet taste sensitivity. Diurnal variation of leptin levels and sweet recognition thresholds may be associated with overall efficacy of insulin on blood glucose.

Poster session I Poster #63

Regulation of the *Drosophila* coreceptor

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Insect odorant receptors (ORs) are heteromeric assemblies of a conventional OR protein, responsible for odorant recognition and Orco, a ubiquitously expressed coreceptor protein. In heterologous systems, Orco alone is capable of forming functional channels, which are unresponsive to odorants. Although Orco has been extensively studied, little is known about single channel properties and its regulation. Here, we show by electrophysiological recordings that both cAMP and VUAA1 can activate Orco. VUAA1, however, was able to activate Orco faster that cAMP. The dose-response curve revealed an EC50 value of 0.7 nM for cAMP and a Hill coefficient of 0. 40. Interestingly, increasing cAMP concentration led to larger unitary single channel current suggesting that Orco might be synchronized by cAMP. The sensitivity of Orco to cAMP was significantly reduced after dialysis with the G-protein inhibitor GDP-β-S, and upon inhibition of phospholipase C (PLC) (U73122) or protein kinase C (PKC) (Gö6976). Stimulation of PKC by phorbol myristate acetate (PMA) or OAG, a diacylglycerol analog caused an activation of Orco even in the absence of cAMP. Furthermore, mutation of five putative PKC phosphorylation sites in Orco abolished the sensitivity to cAMP. These results provide for the first time insight into basis of Orco channel regulation in vitro. This study was supported by the Max Planck Society.

Poster session II Poster #350

Importance of umami-taste sensation. Part 2: loss of appetite and weight on elderly due to umami-taste disorder

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Patients with taste disorder often complain of persistent impairment of palatability related to umami-taste, although the other four basic taste sensations (sweet, salty, sour, bitter) are normal, or even after these four basic taste sensations have been improved by clinical treatment. At present, it is unknown whether such patients' complaints relate to the loss of umami-taste sensation, since there is no clinical test for assessment of umami-taste sensitivity. Therefore, we decided to develop a clinical umami-taste sensitivity test similar to the existing filter paper disc (FPD) method being used for the four basic taste tests other than umami. We used monosodium glutamate (MSG) as an umami solution with six levels of concentration, *i.e.*, 1, 5, 10, 50, 100 and 200 mM MSG.

We clinically applied the FPD methods including umami to the patients who visited our clinic complaining of taste disorder. Recognition threshold (RT) of the umami sensation to MSG was compared to healthy volunteers with normal RT. In seven out of forty-four patients (16 %), RT for umami-taste only was higher than that in healthy volunteers, whereas the other four basic tastes were all within normal levels. Those patients with loss of umami-taste sensitivity were all over 65 years old, and they all complained of appetite loss and weight loss, resulting in poor general health. After treatment, the RT for umami-taste of the patients returned to normal levels, and then they recovered their subjective umami-taste sensation. Concomitantly, they also remarkably regained their appetite and weight. The patients were pleased with regaining of taste sensation and health.

These results suggest that retaining umami-taste sensation is important for the elderly to maintain good health.

Poster session II Poster #392

IRF-3 regulates the expression of multiple pathways in taste tissues

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The mammalian taste buds are exposed to the oral cavity through taste pores and, therefore, are susceptible to invasions by various pathogens. Taste bud cells are found to constitutively express many immune response-related genes. Among them are several Toll-like receptor (TLR) genes which play important roles in pathogen recognition and the onset of inflammation. To further understand the roles of TLR signaling pathways in taste tissues, we carried out experiments to identify the genes whose expression is regulated by constitutive TLR signaling. We used a mouse strain that has a functional deletion of the interferon regulatory factor-3 (IRF-3), a key transcription factor that mediates the signal transduction of several TLRs. Total RNAs were prepared from foliate and circumvallate epithelia of IRF-3-deficient mice and wild type control mice. RNAs were reverse-transcribed into cDNAs which were then amplified and used for genome-wide gene expression analyses using Illuminia bead arrays. This study identified 249 genes whose expression was significantly up- or down-regulated in taste tissues of IRF-3-deficient mice compared with control mice. Although some of these genes are clearly related to immune responses, many others have diverse functions and are involved in multiple cellular and physiological pathways. Quantitative real-time RT-PCR and immunostaining experiments were performed to further evaluate the expression of these identified genes in taste papillae. In summary, our study shows that IRF-3, a key mediator of TLR signaling, regulates the expression of numerous genes in the peripheral taste tissue and many of them have not been previously recognized as IRF-3 target genes. This study is supported by NIH grants DC010012, DC007487, T32DC000014, and P30DC011735.

Poster session II Poster #64

Chemosensory mediated pre- and post-mating reproductive barriers between two moth species, *Spodoptera littoralis* and *Spodoptera litura*

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Understanding the origin of pre- and post-mating reproductive barriers that reduce gene flow among populations is the essence of the biological species concept. Because reproductive barriers can occur in various forms it is crucial to identify processes that lead to reproductive isolation. Focusing on the chemosensory-based behaviors and the chemical signals involved, we quantified potential pre- and post-mating reproductive barriers between two closely related moth species, *Spodoptera littoralis* and *Spodoptera litura*. We examined both pre-mating (sexual behavior, female-emitted sex pheromone, male attraction) and post-mating (mating frequency and latency, oviposition rate, hatchability of eggs, longevity, hybrid viability) barriers between *S. littoralis* and *S. litura*. Our results revealed no clear differences in the temporal aspects of female calling behavior. However, we observed both qualitative and quantitative differences in the pheromone composition of the two species. Despite these differences we observed a robust con- and hetero-specific male attraction in flight tunnel experiments. In females we saw that mating triggered dramatic changes in the behavior and reproductive physiology. Significant differences in the female longevity and fecundity were found as a function of con- and hetero-specific mating. The findings of this study highlight the evolution of multiple chemical-mediated reproductive barriers that entail reduced reproductive success between two moth species.

Contributed talks II "Gustation" Monday 25 June

Sugar aversion in the German cockroach is mediated by changes in gustatory sensillum function

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Glucose is a universal phagostimulant in many animal species, including cockroaches. However, some populations of the German cockroach (*Blattella germanica*) are behaviorally deterred from eating glucose. It is thought that the "glucose-averse" (GA) trait has evolved in response to toxic baits containing glucose. Although GA cockroaches incur significant fitness costs in normal foraging on insecticide-free foods, this trait confers greater survivorship under the strong selection pressure of bait-based pest control. To understand the mechanisms that underlie glucose aversion, we characterized the electrophysiological responses of gustatory neurons involved in glucose reception. Glucose and fructose elicited neural responses from the sugar receptor neuron in gustatory sensilla on the paraglossae of wild-type cockroaches (WT), while caffeine elicited responses from the bitter receptor neuron. On the other hand, while fructose also elicited responses from the sugar receptor neuron in the GA cockroach strain, glucose elicited responses from both the sugar and bitter receptor neurons. Our results suggest that the GA cockroaches may express a glucose reception system(s) on the bitter receptor neurons of the paraglossae.

Generally, feeding behavior is elicited through activation of sugar receptor neurons, whereas the activation of bitter receptor neurons suppresses feeding behavior and evokes aversive behaviors. Our results indicate that different sensory inputs in glucose reception result in opposite foraging behaviors in WT and GA cockroaches. Sensory mechanisms underlying the evolution of behavioral resistance have been enigmatic, and glucose-aversion in cockroaches has emerged as an excellent model system to further our understanding of how the peripheral sensory system can adaptively respond to environmental pressures, such as insecticides, with adaptive changes that confer behavioral resistance.

Supported in part by the Blanton J. Whitmire Endowment at NCSU.

Poster session II Poster #124

Neural circuits underlying olfactory-encoded behaviours in the mouse brain

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Chemosensory cues influence sexual behavior and reproductive physiology. Yet, how the olfactory system impinges onto different output neurons in the brain that are mediating these effects remains largely unknown. Socially important chemosensory cues converge onto a small subset of neurons that produce gonadotropin-releasing hormone (GnRH). These GnRH neurons project to subsets of neurons in brain areas implicated in olfactory-encoded behaviors, raising the possibility that GnRH is released locally within the central nervous system in response to chemosensory cues and acts on downstream target cells expressing the GnRH receptor (GnRHR). To test this hypothesis we have started to characterize GnRHR neurons in mouse brain slices using a novel mouse strain that expresses GFP after Cre-mediated recombination in these cells. By means of confocal microsopy, GnRHR neurons showed robust GnRH-induced Ca2+ elevations in areas known to influence olfactory-encoded behaviors. These signals differed in their waveform depending on stimulus strength and brain area. Using the loose patch clamp technique, GnRHR neurons responded reproducibly with an increase in spike activity to 1-s GnRH pulses implying that synaptic GnRH release may trigger a response in these neurons. GnRHR neurons differed in their spike activity between hypothalamic nuclei suggesting the existence of specialized subpopulations of GnRHR neurons with different patterns of innervation. We have now started to characterize GnRH-induced response patterns during different female reproductive stages to determine whether they can be modulated by chemosensory cues. Supported by grants from the Deutsche Forschungsgemeinschaft (SPP 1392, SFB 894) and the VolkswagenStiftung.

Poster session II Poster #66

Octopamine controls appetite in fruit flies

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We studied the role of the biogenic amine octopamine in sucrose responsiveness and non-associative learning of fruit flies (*Drosophila melanogaster*). Sucrose responsiveness was investigated using the proboscis extension response to stimulation of the tarsae with increasing sucrose concentrations. Non-associative learning was studied as habituation of proboscis extension to tarsal stimulation with a low sucrose concentration. Flies with the TβH mutation lack the enzyme tyramine β-hydroxylase, which catalyzes the last step in octopamine biosynthesis. They are therefore devoid of octopamine. We show that these octopamine-less flies are significantly less responsive to sucrose than wild-type controls. The reduced sucrose responsiveness directly leads to faster habituation of the proboscis extension response. Systemic feeding of octopamine rescues the sucrose response in the mutants. Interestingly, expressing of TβH / octopamine in only one neuron of the subesophageal ganglion (VUMA4 neuron) using the UAS/GAL4 system raises the sucrose responsiveness of the mutants to normal level. These findings suggest a pivotal role of octopamine in regulating sensory responsiveness to sucrose in fruit flies and imply that the VUMA4neuron is a key player in this process.

Poster session II Poster #164

Short and long term olfactory memory in transgenic mice

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We have used a conditioned odour task to assess learning and memory of mice in multiple experiments over the past 10 years. In this task, mice complete 4-days of discrimination training with S+ and S- odours, followed by a memory test 24 hours later (Schellinck et al., 2001, Chem Senses, 26, 663-72). We have found that despite genetic and phenotypic differences, 13 different inbred strains of mice showed good olfactory discrimination memory performance (Brown & Wong, 2007, Learn Mem, 14, 134-44). Longitudinal experiments with the DBA/2J mouse model of glaucoma were completed with long-term memory tests (3-9 months) and have shown that memory for the discrimination can be maintained for very long periods of time (up to 9 months). Mice that have impaired visual ability tend to perform better than mice with normal visual ability (Wong & Brown 2007, Neurobiol Aging, 28, 1577-93). We have also used the odor preference task to assess age-related changes in olfactory memory in the 5XFAD mouse model of Alzheimer's disease, and have found that 5XFAD mice perform well at 9, 12 and 15 months of age, despite the development of impairments in motor ability and visuo-spatial memory. These results demonstrate that the conditioned odour preference paradigm is a useful tool for investigating long-term olfactory memory in mice. To increase the difficulty of the task, we are currently introducing a memory interference component consisting of exposure to additional odour pairs following discrimination training but prior to memory testing.

Poster session II Poster #67

Behavioural modulation by non-host volatiles in beetles and moths: Semiochemical diversity reduces herbivory

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Habitat and background signals play an important role in insect host orientation. Recently we discovered a new olfactory signal functionality related to host selection of both beetles and moths. In both groups, responses (electrophysiological and/or behavioural) to host plants could be modulated by signals from non-host sources. In mixed habitats, non-host plants volatiles (NHV) are not background noise; instead, they are ecologically significant signals that may interfere with host recognition or orientation of herbivores, lowering the response to kairomones and pheromones, thereby conferring associational resistance to host plants.

The "semiochemical diversity hypothesis" (SDH), coined by Zhang & Schlyter (2003), is now substantiated across insect taxa. The non-host volatiles in a biodiverse habitat, like a mixed forest, disturb insect host and mate location. For conifer-inhabiting insects SDH has been exploited for insect control in the field by dispensing synthetic NHV semiochemicals to create "artificially mixed" forests resulting in lower insect infestations. Two examples, one for a pine moth (Jactel et al. 2011) and one for a bark beetle (Schiebe et al. 2011) are given.

For the specialist conifer-feeding bark beetle *Ips typographus,* we have found both a high fraction of olfactory sensory neurons (OSN) responding to NHV molecules and an inhibition of response in a pheromone OSN when a NHV-responsive cell co-localised in the same sensillum was stimulated.

Interestingly, in moth species displaying a range of different dietary widths -from the polyphagous to the monophagous- evidence is found for the importance of NHVs in ecology, behaviour, or sensory physiology. In our polyphagous model

species, *Spodoptera littoralis*, the physiological and molecular mapping of OSN diversity will provide insight regarding odour coding for host and non-host recognition in this species, and through comparison, with beetles and other moths.

Poster session I Poster #307

Amygdala response to male anxiety chemosignals in anxious versus calm women

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Chemosensory signals evoke a behavioral, hormonal, and neural response in healthy humans although the neurobiological substrate is unknown. Humans communicate their emotional situation not only by means of visual and auditory stimuli but also using chemosignals. The current study aimed to investigate the neural correlates of chemosignals during intersexual communication and the influence of personality traits on cortical processing of these cues. Ten healthy male subjects donated anxiety sweat by performing a validated anxiety-inducing task in a high rope course. Emotionally neutral sweat was collected during stationary bicycle training. Both chemosignals were presented to

14 healthy female subjects using constant-flow olfactometry during two fMRI sessions (anxiety/emotionally neutral) in a pseudo randomized order carried out on a 3T MRI scanner. Functional and structural data were evaluated with the help of Matlab and SPM8. Effects of the stimuli with respect to trait anxiety were assessed using Spielberger's State and Trait Anxiety Inventory (STAI-X2) scores and two-sample t-tests. The two chemosensory stimuli were not perceived differently regarding intensity and pleasantness. We were able to demonstrate that the amygdala, a typical emotion processing brain region, responds to a higher extent to chemosensory anxiety signals in less anxious women compared to more anxious women. On the one hand, these results might be explained by a higher basic level of activation in the involved brain regions of more anxious women, which remains stable when exposed to chemosensory anxiety cues. On the other hand, chemosensory anxiety cues achieve to enhance activation of these structures in less anxious women. This study provides evidence for the influence of trait anxiety on the cortical processing of chemosensory cues.

This research was funded by a start up grant from the Medical Faculty of the RWTH Aachen.

Poster session II Poster #222

Control of reserve stem cell activation in the olfactory epithelium

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The mammalian olfactory epithelium (OE) has multiple robust stem cell populations that underlie the tissue's ability to both replenish and recover after injury. Two populations of multipotent stem cells have been identified in the OE based on location, morphology, molecular profile, and functional capacity. Globose Basal Cells (GBCs) lie immediately basal to the immature neuronal layer and constitute a heterogeneous population of lineage-committed and uncommitted progenitors. GBCs closely resemble the original embryonic progenitors of the olfactory placode. Transplantation, lineage tracing, and molecular profiling have shown that GBCs are the predominant stem cell population responsible for neuronal replacement within the OE. By contrast, Horizontal Basal Cells (HBCs) are the basal-most cells in the OE and resemble the basal stem cells of other epithelia by morphology, location, and marker expression. However, HBCs do not function as multipotent progenitors unless the OE as a whole is injured severely. In addition, HBCs appear later in development from a GBC-like cell type, demonstrating that they are not essential for the de novo formation of the tissue. Thus, HBCs function as a reserve stem cell population that requires activation, while GBCs are the predominant homeostatic stem cell population. Cooperation of this type between two stem cell populations raises the basic question: how is the transition

from reserve stem cell to active participant in tissue regeneration and subsequent return to dormancy regulated? In this study we demonstrate a role for the transcription factor p63 in the regulation of HBC activation using conditional knockout and retroviral overexpression techniques. We also present an elegant system for labeling and lineage tracing HBCs for use in genetic manipulation, drug testing, transplantation, and transcription profiling of HBCs-.

Poster session I Poster #87

Oviposition site selection and chemosensory cues in two African malaria vectors

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The current knowledge on olfactory mediated host-seeking behaviour in mosquitoes is extensive, and has recently even reached the molecular level in some species. In contrast, odour cues for oviposition site selection have received limited attention, especially in the malaria vectors. Here, we present data on behavioural responses and electrophysiological correlates of gravid females to oviposition cues in two main African malaria vectors, *Anopheles gambiae* and *A. arabiensis.* We demonstrate that olfactory cues play a role in oviposition site selection for these species. Taken together with what is known from the literature we confirm previous speculations on the complexity and high variability of both the composition of oviposition site volatiles and the gravid females' oviposition choice towards these bouquets. The variability indicates that the oviposition site selection behaviour of gravid females probably cannot be explained with such simplicity as has been shown for host seeking, and/or that odour cues might not be as important for oviposition site selection as they are in host finding. We analyzed the odour bouquets of typical breeding sites using headspace volatile collections, and we are currently verifying the responses of olfactory receptor neurons to these bouquets using gas chromatography coupled single sensillum recordings. We will show our first results from the ongoing screens, which are conducted to narrow down and identify behaviourally active oviposition site compounds and compound classes.

Poster session I Poster #37

Expression pattern of 'Plus-C' class odorant binding proteins in the antenna of the malaria vector *Anopheles gambiae*

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In females of the malaria mosquito *Anopheles gambiae* (Ag) olfaction plays a crucial role in various behaviours, most strikingly in seeking after a blood meal and finding of oviposition sites. The first step of odorant recognition in chemosensory sensilla on the antenna involves soluble odorant binding proteins (OBPs), which transfer odor molecules to olfactory receptors (ORs) in the dendritic membrane of olfactory sensory neurons. Of the three OBP-classes, which have been identified in *A. gambiae* members of the 'Plus-C' class are characterized by the 'classic' OBP core with sequence extensions containing additional cysteines forming further disulfide bridges. Recent RT-PCR experiments and microarray studies have shown that two types of 'Plus-C' class OBPs, called AgOBP47 and AgOBP48 have abundant transcripts in female antennae and are partially down-regulated after a blood meal, suggesting a possible role in the detection of host odorants. By means of whole mount fluorescence in situ hybridization (WM-FISH), we have visualized the AgOBP47 and AgOBP48-expressing cells in female and male antenna, explored their antennal topography and determined their position relative to cells that express 'classic' OBPs and AgORs as well as the AgOR co-receptor (AgOrco). For both AgOBPs a much higher total numbers of expressing cells were detected in female compared to male antennae. AgOBP47 and AgOBP48 are expressed in support cells of distinct sensilla types, with AgOBP48 present in very high number of

sensilla. Furthermore, by applying two-color WM-FISH, it was found that cells which express either AgOBP48, AgOBP1 or AgOR1 are housed together in certain sensilla, indicating that an interplay of the proteins may contribute to the specific responsiveness of the sensillum to distinct odorants.

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Poster session II Poster #192

Formyl peptide detection by vomeronasal sensory neurons is mediated by functionally distinct cell populations

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Several pathogen-derived peptides have been recently discovered as a new class of agonists for vomeronasal sensory neurons (VSNs). In the immune system, these peptides are recognized by formyl peptide receptors. Moreover, a subset of VSNs that express formyl peptide receptor related receptors (Fpr-rs) has been newly identified. These receptors are most likely involved in recognition of pathogen derived and immune modulatory peptides by the VNO. To assess the physiological and molecular mechanisms underlying the detection of these peptides we established a novel high-throughput calcium imaging assay for the automated functional analysis of large numbers of individual, dissociated VSNs. This method was used to test more than 11,000 VSNs, obtained from OMP-GFP mice with classical formyl peptide receptor agonists. We observed several neuron populations that exhibit different pharmacological profiles. Our studies revealed a population of neurons that show an agonist profile corresponding to that of a heterologously expressed Fpr-receptor. Altogether, up to 15% of the excitable neurons could be activated by Fpr-ligands. More than 50% of these neurons responded to several Fpr-agonists. Surprisingly, a subpopulation of these cells was also activated by classical agonists for type 2 vomeronasal receptors (V2Rs). Given that Fpr-rs receptors are expressed in approximately 4% of the VSNs, we assume that other receptors, most likely V2Rs, contribute to the detection of formyl peptides. Thus sensing of formyl peptides by the VNO seems to be more complex than previously thought. To assess the underlying molecular mechanisms we are currently examinig VSNs from several genetically engineered mice with labeled or modified signal transduction components. Supported by DFG-grants SFB894, INST 256/273-1 FUGG and Volkswagen Foundation.

Poster session I Poster #65

Olfactory behaviour of butterflies with divergent host plant range

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In general, moths are considered to be mainly olfactory and butterflies mainly visual animals. However, the olfactory system in Lepidoptera is well developed and similar across butterflies and moth. Within the butterfly family Nymphalidae, host plant range is rather broad with monophagy and wide polyphagy even in closely related species. How these species specific degrees of host- specialization are reflected in the perception and use of olfactory information is poorly known. A recent study in our research group demonstrated that two species of nymphalid butterflies possess neural capabilities to respond to host-plant related odorants. The two species showed similar glomerular activity patterns, although a species-specific difference in sensitivity and correlation between responses to common green leaf volatiles was found. We suggested that these butterflies have the ability to detect and discriminate between plant related odorants. In addition, the data indicated that the specialist *Aglais urticae* appeared to be more discriminative towards odours than the closely related generalist *Polygonia c-album*. In order to test this, behavioural two-choice experiments were performed. The specialist picked out the host plant odour when opposed to a non- host – even blending the host with non-hosts did not impair finding the host. The generalist on the other hand did not show as precise abilities even though it seems it preferred a mixture of several over a single host. Thus, the two species differed in their behavioural responses to the host plant related odorants.

Symposium 16 "Taste and beyond - integration of nutrient sensor functions in oral cavity and gut - Ajinomoto Symposium" Tuesday 26 June

Role of gut nutrient sensing in stimulating appetite

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Food appetite and preference are influenced by the flavor (taste, odor, texture) of foods but also by the post-oral actions of nutrients. This is demonstrated by studies in which the intake of a flavored solution is paired with an intragastric (IG) nutrient self-infusion (e.g., glucose, corn oil, glutamate), and a different flavored solution is paired with a water infusion. Appetite stimulation is revealed by increased intake of and preference for the flavor paired with the nutrient infusion. Flavor preference conditioning can occur in a single training trial, in non-deprived as well as food-deprived animals, and is very resistant to extinction. The magnitude of the conditioned preference varies as a function of flavor quality, nutrient type and energy density. The upper small intestine is a primary site of action for glucose conditioning as indicated by the effectiveness of duodenal and jejunal infusion sites, but not by infusions that bypass the upper intestine (ileal or hepatic-portal). The identity of the nutrient sensors that mediate flavor conditioning is not known. However, gut sweet "taste" signaling is not critical for because T1R3, gustducin, and TRPM5 knockout mice show robust flavor conditioning to IG sugar infusions. Rather, a glucose-specific sensor is suggested by the conditioning response to glucose but not fructose or sucralose. Also unknown is the gut-brain pathway that mediates sugar and fat conditioning. Vagal deafferentation blocks glutamate but not carbohydrate or fat conditioning. This suggests hormonal mediation but currently identified gut hormones are not likely candidates for nutrient-induced appetite stimulation. Post-oral sugar and fat sensing activates the brain dopamine reward system that modulates food preferences.

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Plenary lecture Wednesday 27 June

Taste recognition in *Drosophila*

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The ability to identify food that is nutrient-rich and avoid toxic substances is essential for an animal's survival. Although olfaction and vision contribute to food detection, the gustatory system acts as a final checkpoint control for food acceptance or rejection. The fruit fly *Drosophila melanogaster* tastes many of the same stimuli as mammals and provides an excellent model system for comparative studies of taste detection. The relative simplicity of the fly brain and behaviors, along with the molecular genetic and functional approaches available in *Drosophila*, allow the examination of gustatory neural circuits from sensory input to motor output. We have utilized a combination of molecular, behavioral, and calcium imaging studies to determine the taste ligands that different gustatory neurons detect. These studies demonstrate that taste cells are tuned by taste category and are hardwired to taste behaviors, supporting the labeled line encoding of taste information. Current studies combine behavioral screens and anatomical approaches to identify higher-order neurons that participate in taste processing.

Poster session I Poster #251

Orbitofrontal grey matter volume predicts olfactory acuity in healthy subjects

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Recent studies suggest an important role of higher-order olfactory brain areas in determining basic olfactory performance, but cortical anatomical markers for the variability in olfactory perceptual acuity among healthy subjects have not yet been explored. In the present study, we addressed this question by correlating voxel-based morphometry data, obtained from

90 healthy adults' cerebral anatomy, with olfactory performance measures. In a region of interest (ROI) analysis of functionally defined olfactory cortical regions and olfactory bulb volume, we further sought to disentangle the relative contribution of volumetric differences in central and peripheral areas to behavioral variability. Significant whole brain correlations of grey matter (GM) volume and the combined olfactory threshold, discrimination and identification (TDI) score were observed around the right orbital sulcus. Using the ROIs for anatomical restriction, a multiple regression analysis confirmed this effect on TDI scores and indicated a trend for an association with olfactory bulb volume. Looking at the behavioral subscores separately, a functional division emerged: for olfactory threshold and discrimination, an association with orbitofrontal GM volume, but not olfactory bulb volume and piriform GM volume, was observed. On the other hand, for identification, we found that olfactory bulb volume, but not any of the cortical measures, explained the behavioral variance. In line with previous clinical studies, our study thus suggests an important role of regional GM volume in the right orbitofrontal cortex for perceptual acuity during olfactory processing. Piriform cortex volume did not predict olfactory processing abilities. The observed differences between behavioral subscores in the relative contribution of peripheral and central structures could offer interesting opportunities for delineating a model of olfactory processing which merit further scientific attention.

Poster session II Poster #252

The role of odorant structure in inter-individual variability in odor hedonic perception

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In humans, smells are usually first described by their hedonic character. For some smells, hedonic appreciation may vary significantly between individuals whereas other smells obtain higher hedonic agreement between subjects. In the present psychophysical study, we set out to examine whether odorant structure could account for some of this inter-individual variability. To this end, 30 human volunteers, averaging 22 years of age (22.63 ± 4.32 years; 15 women) were stimulated with 54 odorants. Subjects were to estimate intensity, pleasantness, familiarity, edibility of the stimuli using a scale from 1 (not at all) to 9 (extremely). The first result of interest was that the 54 odorants differed in their hedonic profile: for 27 odorants, the majority of participants agreed in judging them either pleasant or unpleasant (binomial low, $p<0.01$ for all 27 stimuli); for the 27 remaining odorants, a larger hedonic variability was found between individuals (the same molecule was rated as pleasant by about 50% of the subjects and unpleasant for the other 50%). The second result of interest was that the complexity of odorant structure accounted for this hedonic variability between odorants: simple molecules induced higher agreement between individuals than complex molecules ($p<.006$).

Taken together, these findings suggest that beyond learning and gene expression, two factors that previous studies showed to contribute to this hedonic variability, complexity of odorant structure is a prominent determinant of inter-individual agreement in odor hedonic judgement.

Poster session II Poster #308

Non-invasive study of the human stress response to odor

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In modern societies, “stress” is one of the important issues in the public domain. Not only working adults, but even school age children feel stress, especially that caused by interpersonal problems. Stress causes physiological changes like insomnia. Thus we see many products and remedies being developed for the purpose of stress relief. Since ancient times, lavender oil has been employed in aromatherapy as one of the most common remedies for stress. In this study we sought to gather evidence of the benefits of lavender aroma in relieving daily stress, examing how olfactory stimulation from lavender oil can modulate human emotional stress responses.

To mimic emotionally stressing daily office tasks in a busy office, healthy female volunteers were asked to memorize information from a foreign language within a time limit. We performed serial non-invasive measurements such as blood pressure, heart rate, visual analog scale and saliva amylase. Saliva amylase is considered a good stress marker. We can collect saliva amylase directly from volunteers' mouths and immediately obtain data on the point. Surprisingly, we discovered different patterns of emotional stress responses in our subjects those are different from our previous data of emotional stress responses obtained from the hypothalamus of rats. Further studies are planned to measure blood stress peptides like vasopressin and cortisol.

Poster session II Poster #312

Clinical diagnosis of the olfactory nerve transport function

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Nasal administration of macromolecular drugs (peptides, nanoparticles) has a possibility to enable drug delivery system beyond blood brain barrier via olfactory nerve transport. Basic research for drug deliver system to brain with nasal administration has been well studied. However, the olfactory nerve transport dysfunction in patients with olfactory disorders is to be determined for the choice of candidates in clinical trial. Nasally administered thallium-201 is transported to the olfactory bulb, as has been shown in healthy volunteers. Furthermore, transection of olfactory nerve fibers in mice significantly decreases transport of nasally administered thallium-201 to the olfactory bulb. The olfactory nerve transport function was reduced in the patients with impaired olfaction due to head trauma, upper respiratory tract infection, and chronic rhinosinusitis relative to the values in healthy volunteers in this study. Furthermore, the olfactory bulb volume was assessed on MRI image to determine a cause of reduced olfactory nerve transport dysfunction in the patients with hyposmia due to chronic rhinosinusitis that is classified in respiratory hyposmia. Both odor thresholds and olfactory bulb volume were significantly correlated with the olfactory nerve transport function in the subjects. Those results suggest the hypothesis that olfactory nerve transport dysfunction may effect on the decrease of olfactory bulb volume in the patients with olfactory disorders. We would also discuss the relationship between the regeneration of olfactory nerve transport function and olfactory bulb volume in the patients with olfactory disorders.

Poster session I Poster #351

Angiotensin II signaling modulates taste responsiveness in mice

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Modulation of gustatory function critically influences food preference, food intake and metabolic homeostasis. Even so, the mechanisms for modulating taste sensitivity are poorly understood. Here, we report that angiotensin II (AngII), which plays important roles in the maintenance of sodium and water homeostasis, modulates salt and sweet taste sensitivities in mice. The chorda tympani nerve recording demonstrated that AngII suppresses amiloride - sensitive taste responses to NaCl. Surprisingly, AngII enhances sweet taste responses, without affecting responses to KCl, sour, bitter and umami tastants. These effects of AngII on gustatory nerve responses are blocked by an AngII type1 receptor (AT1) antagonist. Behavioral tests showed that lick rates (per 10sec) for NaCl and sweeteners are significantly reduced by the AT1 antagonist in water deprived mice. Expression analyses revealed that AT1 are co-expressed with αENaC (amiloride - sensitive epithelial sodium channel α-subunit: a salt taste receptor) or T1r3 (a sweet taste receptor component) in a subset of taste cells. These results suggest that taste organ is a new peripheral target of AngII, and the specific modulation of amiloride - sensitive salt and sweet taste sensitivities may play important roles in regulating sodium and glucose homeostasis.

Poster session I Poster #223

Sprouty2 is required for maintenance of the olfactory epithelium

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During embryogenesis, Fibroblast Growth Factor (FGF) signaling plays a key role in promoting formation and invagination of the olfactory placode, and in generation of olfactory epithelial cells (Bailey et al. 2006, Kawauchi et al. 2005, Maier et al. 2010, Sjödal et al., 2007). At postnatal stages, FGFs are used extensively to culture the olfactory epithelium (Barraud et al. 2007, Krolewski et al. 2011, Murrell et al., 2005), however, the in vivo role of FGF signaling in the postnatal olfactory epithelium is unclear. The Sprouty gene family encodes antagonists of receptor tyrosine kinase signaling, including FGF signaling. We find that a mouse knock-out of the Sprouty2 (*Spry2*) gene results in a progressive, postnatal degeneration of the olfactory epithelia: the majority of *Spry2* mutants between 3 to 5 weeks of age have histologically normal olfactory epithelia, whereas in a majority of *Spry2* mutants older than 8 weeks of age, the olfactory epithelia are severely disorganized and thinned. Preliminary immunohistochemical analysis suggests that there is no gross defect in specification of the various cell types of the olfactory epithelium in young, 3 to 5 week-old *Spry2* mutants. We hypothesize that the degeneration of the epithelium in *Spry2* mutants is due to either an increased susceptibility of the olfactory epithelia to damage or a decreased ability to recover from environmental damage over time. Experiments that both address this hypothesis and investigate the genetic relationship between *Spry2* and FGF signaling in the olfactory epithelium will be presented.

Symposium 16 "Taste and beyond - integration of nutrient sensor functions in oral cavity and gut - Ajinomoto Symposium" Tuesday 26 June

Sensing of amino acids by the gut expressed T1R1-T1R3 leads to cholecystokinin (CCK) secretion.

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The satiety controlling hormone, CCK is secreted from enteroendocrine I cells in the upper small intestine in response to dietary fat, protein, peptides, or amino acids. The aim of this study was to identify if the gut expressed T1R1-T1R3 (umami receptor) is the initial signal recognition site for these nutrients. Using immunohistochemistry, we showed that T1R1, T1R3, α-gustducin and CCK are co-expressed in enteroendocrine cells of the mouse (and pig) proximal small intestine. Employing RT-PCR, we confirmed that STC-1 cells (a murine endocrine cell type) also express T1R1, T1R3, α-gustducin and CCK. We demonstrated that STC-1 cells release CCK in response to their exposure to phenylalanine (PHE), leucine (LEU), monosodium glutamate (MSG), glutamate tripeptide and hydrolysates of meat protein, albumin egg and soy protein. Using the technique of RNA interference, we inhibited (>60%) the mRNA expression of T1R1 in STC-1 cells. The inhibition of T1R1 expression had no effect on the ability of STC-1 cells to secrete CCK in response to protein hydrolysates, and the glutamate tripeptide. In contrast, CCK secretion was significantly decreased in T1R1 'knock-down" STC-1 cells in response to amino acids PHE, LEU and MSG. Inclusion of inosine 5'-monophosphate (a specific potentiator of T1R1-T1R3 signalling) resulted in a further increase in CCK secretion by STC-1 cells in response to individual amino acids. Moreover, exposure of everted mouse proximal small intestinal segments to PHE, LEU and MSG led to significant increases in CCK release compared to untreated controls. CCK secretion by tissue segments in response to amino acids was dramatically decreased by pre-incubation of tissue segments with gurmarin. Collectively, the data support that T1R1-T1R3 receptor is involved in the secretion of CCK in the proximal intestine in response to amino acids.

Poster session II Poster #254

Odorant induced Gamma-band activity in the human olfactory epithelium

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The olfactory system presents a unique opportunity to directly record neural activity in humans in vivo. Olfactory receptor neurons (ORNs), a form of PNS-CNS transition neuron outside the skull, located in the olfactory epithelium, enable in vivo recording of olfactory responses from awake behaving humans. To date, local field potentials (LFPs) referred to as electro-olfactograms (EOGs) have been analyzed in the time domain. This typically included event-related computations of either amplitude or area under the curve of the EOG response. Here we set out to probe for odorant-induced responses in the frequency domain. We used endoscopic guidance to place an Ag/AgCl electrode coated with Teflon tubing (0.8 mm OD) filled with Ringer-agar (1%) on the middle turbinate. A computer-controlled olfactometer delivered an odorant (Ethyl 3-methyl-3-phenylglycidate, CAS# 77-83-8) into the recorded nostril via Teflon tubing (inner diameter = 2.15 mm), maintaining steady mechanical and thermal conditions (5.5 SLPM, 37 °C, 80% RH). Stimulus duration was 0.5 s, and inter-stimulus-interval was > 25 s. The experimental paradigm contained 27 repetitions without nasal inhalation. Data was recorded at 1 KHz. We aligned the responses in time, and plotted the power over frequency of response. This revealed a clear odorant induced response at the Gamma-band range, between 30 and 80 Hz, that occurred between ~400 to ~800 ms post odor onset. This approach may allow investigation of odor-specific response profiles in the frequency domain.

Poster session I Poster #397

Heterologous overexpression of functional gurmarin, a sweet-taste-suppressing protein, by the methylotrophic yeast Pichia pastoris

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Gurmarin is a polypeptide isolated from the leaves of *Gymnema sylvestre*. This peptide is made of 35 amino acid residues, including three disulfide bridges and an amino-terminal pyroglutamyl residue. Gurmarin is known to selectively inhibit responses to sweet substances in rodents without affecting responses to other basic taste stimuli, such as NaCl, HCl, and quinine. Recent studies have proposed that gurmarin may interact with rodent T1R2/T1R3 sweet taste receptors. Here we report the heterologous expression of gurmarin using the methylotrophic yeast *Pichia pastoris*. Gurmarin was secreted into the buffered minimal medium using the α-factor preprosequence without the EAEA spacer peptide of *Saccharomyces cerevisiae* and was under the control of the methanol-inducible alcohol oxidase promoter. We found that gurmarin accumulated in the yeast culture medium reaching 5 mg per litre of culture over an expression period of 4 days. To compare the production level and the signal peptide processing, the N-terminal amino acid of gurmarin was substituted by a glutamic acid residue. This construct resulted in a 6-fold increase in the level of gurmarin secretion leading to 30 mg of purified protein per litre of culture. The molecular mass of the purified gurmarin was shown by MALDI-ToF mass spectrometry to correspond to that expected for fully reduced gurmarin. Circular dichroism and NMR spectroscopy revealed that recombinant gurmarin is properly folded and has secondary and tertiary structures. We also confirmed its capability to inhibit the rat sweet taste T1R2/T1R3 receptor by functional expression in human embryonic kidney (HEK)293T cells. To the best of our knowledge, this study describes the first recombinant expression of gurmarin. The high level of fully active gurmarin with functional properties identical to those previously described for natural gurmarin opens the door to study the interactions between gurmarin and rodent sweet taste receptors.

Poster session I Poster #33

Olfaction in a disease vector fruitfly, *Phortica variegata*

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The primitive fruitfly, *Phortica variegata* belonging to the family of Drosophilidae and to the Steganinae subfamily, shows a peculiar zoophilic behavior unlike other fruitflies of the same family. Apart from feeding on fruits, *P. variegata* flies also feed on lachrymal secretions of animals and humans thus transmitting *Thelazia callipaeda* (Spirurida, Thelazidae) eyeworms that causes thelaziosis, potentially leading to blindness. What contributes to this special zoophilic behavior in these flies remains unknown. Since olfaction plays an important role in the life of other pathogen transmitting vector insects, we investigated the olfactory system of these flies to understand its role in their distinct zoophilic behavior.

In this study, for the first time, we describe the olfactory system of a vector fruitfly. We show that *P. variegata* flies have well-developed olfactory system similar to their well-known counterpart *Drosophila melanogaster*. In *P. variegata,* the peripheral olfactory organs, the antenna and maxillary palp, harbor distinct types of olfactory sensilla. The primary olfactory center in the brain, the antennal lobes, shows a glomerular organization and receives neuronal input from the peripheral olfactory organs in an organotopic pattern. Further, using electrophysiological methods we demonstrate the ability of these flies to detect both fruit related and animal related odors. These results allow us to compare and discuss the olfactory system of *P. variegata* with that of the well described *D. melanogaster* olfactory system.

Poster session I Poster #69

Mammalian aversion to insect chemical defense compounds

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One of the ways insects try to protect their territory or avoid predation is by releasing defensive chemicals. A wide variety of insects use the same chemicals for this purpose. We hypothesize that defensive insect chemicals used by multiple insect orders are targeting the mammalian trigeminal system and eliciting chemesthesis. In the current study, we test a number of insect defense compounds (benzaldehyde, benzoquinone, formic acid, 2-Heptanone, 6-methyl-5-hepten-2-one, tetradecane, trans-2-hexenal, and trans 2-hexen-1-ol) that are released by multiple insect orders to determine if they are irritating to a mammalian predator (rat). We first determined if these defense compounds activated the trigeminal nerve by recording from the ethmoid branch while perfusing stimuli through the rat's nasal cavity. We also monitored respiration in the rats both before and after stimuli were introduced as trigeminal nerve activation depresses respiratory frequency. Using these methods, we determined that all of the tested compounds besides tetradecane activated the rat trigeminal nerve. We then determined if rats demonstrated behavioral aversion to these compounds at concentrations released by insects. We placed a rat in a square plexiglass arena with petri dishes in each corner. After a period of habituation, an experimental stimulus was added to one of the four petri dishes and the movement of the rat over a ten minute period was recorded. Ethovision XT (Noldus) was used to analyze whether rats spent less time in the corner containing the irritant. Through this work, we have identified insect defense compounds that could target the mammalian trigeminal system. Our next step is to determine the receptor targets of these compounds using calcium imaging of both primary trigeminal neuron cultures and heterologous expression systems.

Poster session II Poster #68

The role of the *Drosophila* TRPA channels, painless and dTRPA1, in detecting chemical irritants

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The detection of chemical irritants is important for the avoidance of potentially life threatening compounds. In mammals, the trigeminal nerve is an important site of chemical nociception, directly responding to a variety of chemical compounds. One target of irritants is the TRPA1 channel. The fruit fly, *Drosophila*, possesses four homologs of mammalian TRPA1, two of which are painless and dTRPA1. Previous research has provided contradictory evidence regarding the roles of painless and dTRPA1 in fly chemical nociception. We analyzed the behavioral phenotypes of painless and dTRPA1 mutants using the proboscis extension reflex (PER) assay and two-bottle preference test. Both indicate that each channel is required for the behavioral aversion to AITC, though it is not clear whether these channels are acting independently or in combination. We evaluated the expression patterns of painless and dTRPA1 to determine if there was any colocalization. No overlap was observed centrally, and we are currently evaluating potential colocalization peripherally. To further define these cell populations, specific cell markers were identified. We observed subsets of painless and dTRPA1 that were colocalized with the *Drosophila* homologs of mammalian CGRP and Substance P, respectively. Cell excitability of painless and dTRPA1 cells was assessed by employing the GCaMP transgene to observe changes in calcium levels in response to stimulation. Both painless and dTRPA1-expressing cells displayed significant increases in fluorescence following application of AITC. To determine if activation occurred in a direct or indirect manner, painless and dTRPA1 were expressed in an ectopic *Drosophila* tissue. Again, both painless and dTRPA1 demonstrated increases in cell excitability to AITC, suggesting these channels are acting independently to detect irritants. Together, our results provide additional insight into the particular roles of each of these channels plays in chemical nociception.

Symposium 2 "Coding of taste across mammals: from the tongue to the cortex" Saturday 23 June

Neural Activity in taste and reward areas in behaving animals

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One ongoing and controversial question in gustatory physiology is to ascertain how taste with somatosensory input and reward are encoded in various areas of the brain. We have explored this question by recording esembles of neurons in the gustatory cortex, lateral hypothalamus, orbitofrontal cortex , nucleus accumbuns and amygdala in behaving rodents while they were feeding (licking), sleeping and/or making decisions . These areas were recorded alone or in various groupings. Here I will give an overview of current state of knowledge regarding these measurements with regard to broadly and narrowly tuned neurons, coding of intensity participation of neurons in different networks, role of licking (and active tasting) and changes that occur upon learning. This work was supported in part by NIH grant DC-01065.

Poster session I Poster #295

Experience alters unlearned amygdala response to chemosignals

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In rodents, the vomeronasal organ detects both heterospecific and conspecific chemosignals and projects to the accessory olfactory bulb (AOB). The AOB has direct projections to the anterior and posterior divisions of the medial amygdala (MeA, MeP). In mice, MeA immediate-early gene FRAs expression increases for conspecific and heterospecific stimuli. In MeP, conspecific odors increase activation overall, while predatory (but not non-predatory) heterospecific odors activate the ventral division. Hamsters show similar results, but without an increase in MeP activation for predatory (cat) stimuli. In these experiments, animals had no previous experience with chemosignal donors, suggesting the amygdala may be involved in unlearned evaluation of stimulus relevance. To investigate the effect of experience, we used positive reinforcement to change the relevance/salience of a previously neutral heterospecific stimulus (steer urine). We exposed animals to steer urine, female mouse urine and control and examined FRAs activation in medial amygdala and in the mediocaudal division of the intercalated nucleus of the amygdala, m-ICNc, which may be involved in selective suppression of MeP for non-relevant stimuli. Preliminary results in male mice suggest an effect of training on medial amygdala after exposure to both relevant and non-relevant stimuli. The basolateral amygdala is involved in odor learning and activation therein serves as a positive control. The effect of mixing a neutral heterospecific stimulus with components of male mouse urine known to be associated with individual odor learning is also under investigation in female mice.

Supported by NIDCD grant DC005813 and FSU Neuroscience Program grant and fellowship.

Poster session II Poster #272

Perception of a blending odor mixture: An fMRI study in humans

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Odors we perceive from our environment arise from the processing of mixtures of odorants. Some mixtures can lead to configural or elemental perception depending, in part, on experience. However, the neural bases of such influences are still unknown. In the present study, we examined the neurophysiological correlates of the configural and elemental processing of a binary odor mixture (AB). This AB mixture has previously been shown to blend into one percept, but also to produce a more or less configural perception depending on pre-exposure to either the mixture itself or to the single components of the mixture. Therefore, subjects were pre-exposed twice either to AB (group Gmix, n=12) or to its single components (A & B; Gcomp, n=14) in order to favor configural processing in the Gmix group or analytical processing in the Gcomp group. During fMRI, subjects were stimulated with AB, A and B, following a block design, using a computer-controlled olfactometer. At the end of the session, subjects rated intensity, pleasantness, and complexity for each stimulus. Psychophysical data revealed no significant difference in pleasantness but a small difference in intensity (B slightly less intense). Concerning complexity, Gmix subjects perceived the mixture as simple as the components whereas Gcomp subjects perceived the mixture as more complex, revealing experience-induced analytical processing of the mixture for this group. For fMRI data recorded when AB was the stimulus, the contrast Gcomp minus Gmix did not yield any significant differences in activation. However, the contrast Gmix minus Gcomp indicated an increased activation in five brain regions, among which the right superior frontal gyrus, already found to be specifically activated in a PET study on mixtures, and the hippocampus known to be involved in holistic processing of complex visual scenes. These preliminary results shed some light on cerebral mechanisms implied in the processing of complex odors. *Supported by grants from the Burgundy Regional council and EU-ERDF to GC and TTD, European Dijon-Dresden Laboratory (LEA 549) to GC, TH, TTD, and a fellowship from the French MESR to CS.*

Poster session I Poster #379

Psychophysical assessment of taste sensitivity to sodium chloride and monosodium glutamate in mice lacking either the T1R2 or T1R3 receptor protein.

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In taste receptor cells, the T1R3 protein forms a heterodimer with the T1R1 protein or the T1R2 protein that binds with amino acids or sweeteners, respectively. The necessity of the T1R3 subunit in the maintenance of normal detectability of monosodium glutamate (MSG) in mice has been questioned in the literature. Whereas T1R3 knockout (KO) mice have markedly impaired responses to glutamate and sucrose in a brief access test, it has been reported that T1R3 KO mice have normal taste detection thresholds to sucrose and MSG mixed with or without the epithelial sodium channel blocker amiloride, a drug apparently tasteless to rodents that interferes with sodium taste transduction. Recent work in our lab has found that both T1R2 KO and T1R3 KO mice have severe difficulty detecting sucrose. Here the sensitivity of T1R2 KO, T1R3 KO and wild-type (WT) mice to an array of NaCl and MSG concentrations was assessed. Water-restricted mice were trained to discriminate a tastant from water in a two-response operant procedure in which a correct response resulted in access to a water reward and an incorrect response resulted in a time-out. The psychometric detectability functions of both KO genotypes for NaCl (N=5-6/group) and MSG (N=4-6/group) did not significantly differ from WT mice. Apparently, the T1R2 and the T1R3 proteins are individually unnecessary for normal taste detection of MSG and confirm and extend earlier studies with T1R3 KO mice by demonstrating that T1R2 is unnecessary for MSG taste detection despite that MSG shares some taste-related perceptual properties with sucrose in rodents. It is possible that the remaining

T1R subunits could be maintaining competence in this task or that glutamate is activating a T1R-independent mechanism. Alternatively, it is possible that the Na^+ cation is providing a sufficient cue. We are currently testing the latter possibility by determining psychometric functions for MSG mixed with amiloride. NIH R01-DC004574 (ACS) & NSF GRF to KRS

Poster session I Poster #409

Development of a regional taste test that uses edible circles for stimulus delivery

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Measurements of taste function within regions of the tongue surface can yield information on the sensitivity of specific lingual areas to taste stimuli, and may also identify potential nerve damage to the gustatory system. We recently developed a novel regional taste test that is based on edible film technology. Edible films are prepared from a mixture of the polymers pullulan and hydroxypropyl-methylcellulose, poured onto a non-stick surface, and dried. Then, a 5/16-inch hole punch is used to prepare taste circles for testing. Taste circles containing quinine, NaCl, or capsaicin were then used to examine taste intensity and taste quality at 6 different regions of the tongue surface. These regions included the anterior tongue tip, the left and right lateral margins of the tongue, and the middle posterior region of the tongue. Subjects used the general Labeled Magnitude Scale (gLMS) to report a taste intensity response, and then a taste quality response 15 seconds after placing the circle on the open tongue. The experiment was repeated with the mouth closed, followed by having the subject touch the roof of their mouth without moving their tongue. For quinine, the anterior and posterior regions of the tongue yielded the highest values (gLMS $\approx$ 15) when the mouth remained open, and near 25 with the mouth closed. Similar results were obtained with NaCl. The irritant capsaicin activates the trigeminal nerve, which innervates the anterior two-thirds of the tongue. The tip of the tongue responded most strongly to capsaicin, with gLMS values near 25. Lateral regions of the tongue yielded gLMS values near 5. Similar gLMS values were observed for capsaicin with the mouth opened or closed. When blue dye was incorporated into edible circles, almost no spreading of the dissolved taste circle was observed. These results indicate that edible taste circles are an excellent means to examine taste function and chemical irritation on a regional basis. Supported by NIDCD 2R44 DC007291.

Symposium 11 “The stimulus – odor space and chemometrics” Sunday 24 June

Predicting odorant-mixture perceptual similarity from odorant-mixture structure

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A benchmark test for understanding olfaction is the ability to predict important features of olfactory perception from odorant structure. Whereas this benchmark has yet to be attained, a first step in this direction is the ability to use odorant structural features in order to predict perceptual similarity across odorants, regardless of the percept identity. Whereas several recent efforts have attained this goal for monomolecular odorants, the real olfactory world is made of odorant mixtures, and a predictive framework linking such mixtures to perception has yet to be developed. Here we tested two alternative models for predicting odorant mixture similarity from odorant mixture structure. One model considers olfaction as an analytical process, and takes into account all the pairwise distances across all monomolecular components of mixtures. A second model considers olfaction as a synthetic process, and considers each mixture as a whole. We diluted 86 monomolecular components to equal perceived intensity, then generated various mixtures containing 1, 4, 10, 15, 20, 30, 40 or 43 components, and finally conducted 191 different pairwise perceptual similarity experiments in 14 subjects. To generate structural models, we used Dragon software to obtain 1433 physicochemical descriptors for each monomolecular odorant. We found that the analytical model provided a statistically significant but poor link between mixture structure and mixture perceptual similarity ($r=-0.3$, $p < 0.001$). By contrast, despite reliance on reduced information, the synthetic model provided a powerful prediction of perceptual similarity from structure ($r=-0.84$, $p < 0.001$). In other words, we were able to predict the perceptual similarity of complex odorant-mixtures made of equal-intensity components based on the physicochemical structure of their components alone. Methods for optimization of this computation, and its implications for biological models of the olfactory system will be discussed.

Poster session I Poster #309

Regional taste loss disinhibits intact oral sensations and may induce weight gain

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Clinical and laboratory data have revealed health conditions associated with sensory loss in specific regions of the mouth: Severe childhood ear infections (otitis media, OM) damage the chorda tympani (CT) and block anterior taste sensation, while tonsillectomy damages the glossopharyngeal nerve (IX) and blocks posterior taste/tactile sensation. These conditions are linked to long-term obesity risk (e.g., body mass, high-fat food avidity) via unclear mechanisms, but our data implicate shifting interactions among food-related cues. Mounting evidence shows that regional taste loss produces compensatory disinhibition at remaining oral loci; for example, CT anesthesia leads to elevated glossopharyngeal, trigeminal, and whole-mouth sensation, particularly in supertasters of 6-n-propylthiouracil. Recent findings extend this model to oral sensory insults carrying obesity risk. Subjects with medical histories and regional hypogeusia indicating either CT damage (i.e., OM) or IX damage (i.e., tonsillectomy, head/neck radiation) show elevated whole-mouth taste, oral burn and viscosity, and retronasal olfaction (RO). However, subjects with damage to both nerves show reduced whole-mouth taste and RO, failing to counteract extensive loss. These data support previous reports linking RO to oral sensation, underscoring the importance of whole-mouth input in flavor integration. Differential obesity risk with whole-mouth gain vs. loss remains to be seen, but changes in the relative intensity of flavor components appear sufficiently robust to influence high-fat intake. Overall, oral disinhibition sustains whole-mouth taste and flavor sensation by moderating the impact of limited spatial loss, but the strength of this interaction may govern long-term effects on food choice and dietary health.

Poster session II Poster #238

Nose-witness identification

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Each individual has a unique body odor, similar to a fingerprint. For forensic purposes, identification of human body odors has in the past been done with trained dogs and not by humans. Although humans are good at detection and discrimination of odors, we are generally poor at identification body odors and common objects in comparison with visual identification. But, we also know that emotion can aid encoding and later retrieval. We tested whether body odor identification following viewing emotional crime scenes would assume such performance levels that it would be forensically interesting. Participants viewed 1-minute long crime scenes and control scenes which were presented while they smelled a body odor that we instructed to be from the target male character of the scene. After 15 minutes, the participants attempted to identify the target odor in a line-up of 5 body odors. Overall performance was considerably better than chance (20%). Moreover performance for crime scenes was better (68%) than for control scenes (45%). Thus, the results have implications for body odors in forensics, especially in situations where other sensory cues to the identity of the perpetrator may be absent.

Poster session I Poster #253

The effects of body odor in sexual response in humans

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Earlier findings suggest that males rate visual and olfactory information as being equally important for selecting a lover, while females consider olfactory information to be the single most important variable in mate choice (Herz & Cahill, 1997). With this background we investigated if body odors (BO) influence the sexual response in humans. Male and female participants will watch an erotic movie while being exposed to a BO previously collected from a person of the opposite sex or no odor (blank). The subjective sexual response towards the movie will be evaluated and related to the odor condition (opposite sex BO or blank). We expect different effects on the sexual response, depending on the sex of the participant and odor condition. Data collection is undergoing and preliminary results will be presented.

Herz, R. S & Cahill, E. D. (1997) Differential use of sensory information in sexual behavior as a function of gender. *Human Nature , 8*, (3), 275-286.

Symposium 7 "Human olfaction" Sunday 24 June

Like being a rat

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In what is by now an endlessly paraphrased essay, Thomas Nagel asks what is it like to be a bat? In brief, his answer is that we can never really know. Nevertheless, when we try to think of this problem, we typically use our powers of visual cognition. Thus, paradoxically, we don't even approach the question itself in a way a bat likely would. Bat cognition is likely dominated by audition. In turn, most mammalian cognition, save a few odd exceptions, is likely dominated by olfaction. One of the major differences between perceiving the world through the nose versus perceiving it through eyes or ears is in the overall temporal envelope of information. Whereas audition is constant, and vision is rather constant, broken only by blinks and saccades, olfaction is stereotypically quantized by sniffs. These sniffs are highly stereotypical within species, and largely stereotypical across species (4-8 Hz). To try and better understand the impact of this temporal structure on cognition, we built a device that links vision to sniffing. The device included a sniff sensor linked to varying opacity goggles such that when the user sniffs they see, and if they don't sniff, they don't see. So far, one subject wore the device for four days straight, and two subjects wore it each for one day. Daily fMRI revealed remarkable plasticity in both visual and olfactory cortices, the former gaining a reflection of sniffing, and the latter gaining a reflection of visual objects. Behavior rapidly adapted to quantized visual input, allowing near-normal performance of daily activities. This outcome both poses questions on brain-organization of sensory processing, and serendipitously drove the development of a series of sniff-controlled assistive devices for the disabled.

Poster session I Poster #163

High-throughput in-vivo screening of glomerular ligand spectra

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Mice are exceptional in their ability to capture their chemical environment, mapping the olfactory world into a basic sensory representation with over one thousand different types of chemical sensors, that is, olfactory sensory neurons (OSNs). OSNs of each type converge in the olfactory bulb onto exclusive distinct physiological areas called glomeruli. The glomeruli constitute the first relay station of olfactory stimulus representation in the mouse brain. Thus, the stimulus induced glomerular input pattern spatially embodies an important part of the sensory representation in the olfactory bulb. Still, the investigation of these spatial patterns is limited as the ligand spectrum of many glomeruli (their "chemical receptive range") is not known. The number of volatile molecules is vast. Obtaining a representative ligand spectrum for a glomerulus therefore requires sampling the responses to many odors. To tackle this challenge we combined *in-vivo* optical imaging of the neuronal response with robot-assisted odor delivery and iterative virtual odor screening with the aim to detect and quantify glomerular responses with high throughput. As a benchmark for detection quality we determined the responses evoked by more than 100 odors in the location of the MOL2.3 glomerulus, which was genetically stained with GFP. We iteratively enriched the stimulus set by an adaptive sampling approach: After each set of biological measurements, we performed virtual screening for candidate ligands and selected odors for the the next *in-vivo* experiments. In a subsequent step we extracted individual glomerular activation with a novel image analysis approach based on non-negative matrix factorization. This method enabled us to identify non-labeled glomeruli and determined their ligand spectrum. Our aim is to extend our screening approach to identify the ligand spectrum of all glomeruli in the dorsal bulb.

Poster session II Poster #162

A comparison between human perceptions of odor similarity and the gloemrular activity patterns in rats

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Predicting odor qualities is an ultimate challenge for machine olfaction. If this becomes possible, the machine olfaction can be applied as an innovative tool for quantitative evaluation of the odors without collecting a considerable amount of data from the well-trained human panels. Previous studies have proposed two types of approaches to predict human perception. The first type of approach is to convert electric sensor response into the odor qualities by either an engineered regression technique or a bio-inspired signal processing method. The second is to predict odor qualities from over thousand types of the odorant molecular descriptors for chemoinformatics. In addition to these approaches, our research group focused on the internal state in the olfactory system such as the glomerular activity patterns evoked on the olfactory bulb, and took another approach that can predict odor qualities based on these measurement data.

As a first step, we investigated the relationships between the perceptual similarities between different odorants and the glomerular activity patterns evoked by the odorants. Since non-invasive methods for measurement of the glomerular activity patterns from human have not been established, we used the activity patterns measured from rats available online because the basic structure of the olfactory system is common between two species. For the comparison, three indices (correlation, overlap rate of activation, and histogram difference) were calculated between the glomerular activity patterns evoked by two odorants. The comparison results using 15 odorants revealed that the Euclidian distance of the three indices between a pair of odorants has a correlation rate of -0.68 (p<0.01) to the perceptual similarity measured from human panels. We then trained a neural network model to predict the perceptual similarity, and found that the correct prediction rate was about 78%. These results suggest that the use of the internal state model of the olfactory system can be another important approach for the future development of the machine olfaction.

Poster session I Poster #413

Biogenic amines modulate transepithelial potential (TEP) in labellar taste sensilla of the blowfly.

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Biogenic amines act as important neuromodulators, neurohormones and neurotransmitters of many physiological processes in invertebrates (Brigaud et al, 2009). Their effects are observed at various levels of insect nervous systems, such as in modulating the sensitivity of sensory receptors and interneurons (Scheiner et al, 2002). Among biogenic amines, octopamine (OA), structurally related to noradrenaline (Roeder, 1999), tyramine (TA), the biosynthetic intermediate of OA (Brigaud et al, 2009) and dopamine (DA), are widespread in insect nervous tissues (Blenau et al, 2001). Biogenic amines are known to be involved in chemoreception mechanisms, by modulating the transepithelial potential (TEP), for which a major role has been proposed in the generation of the receptor potential (Dolzer et al, 2001; Grosmaitre et al, 2001; Pophof, 2000).

Nonetheless, the effects of OA and particularly TA and DA on taste systems, are little understood. By means of electrophysiological techniques, we investigated whether OA, TA and DA affect the TEP in the labellar taste chemosensilla of the blowfly *Protophormia terraenovae*. Drugs dissolved in saline at 0.1 mM were injected (1 µl) near the labellum base, in order to reach the accessory cells where the K^+-dependent TEP generation mechanism is located (Sollai et al, 2008). TEP values were monitored before and for 20 min (every 5 min) after drug injection.

Results show that OA and TA increase the TEP values already 5 min after injection and throughout the 20 min interval. Conversely, the TEP value is reduced after 5 and 10 min from DA administration, but it rebounds to starting values after 15 and 20 min. Injection of saline (control) does not alter the TEP values.

In conclusion, OA, TA and DA appear to modulate the TEP amplitude, possibly by affecting the electrogenic K^+ transport, as described in other insect epithelia, such as Malpighian tubules in *Drosophila* (Blumenthal, 2005) or olfactory sensilla in *Manduca* (Dolzer et al, 2001).

Poster session II Poster #94

Comparative analysis of deutocerebral neuropils in Chilopoda (Myriapoda): implications for the evolution of the arthropod olfactory system.

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Originating from a marine ancestor, the myriapods most likely invaded land independently of the hexapods. Here, the question arises if and how myriapods have solved the tasks of odor detection and odor information processing in air. Behavioral experiments indicate that chilopods are able to perceive airborne stimuli, both from live prey and prey extracts. Here, we examined the architecture of the deutocerebral brain areas (which process input from the antennae) in eight representatives of the Chilopoda, covering all major subtaxa, by histology, confocal laser-scan microscopy, and 3D reconstruction.

We found that in all species that we studied the majority of antennal afferents target two separate neuropils, the olfactory lobe (chemosensory, composed of glomerular neuropil compartments) and the corpus lamellosum (mechanosensory). The numbers of olfactory glomeruli in the different chilopod taxa ranged from ca. 35 up to ca. 90 and the shape of the glomeruli ranged from spheroid across ovoid or drop-shape to elongate. A split of the afferents from the (first) pair of antennae into separate chemosensory and mechanosensory components is also typical for Crustacea and Hexapoda, but this set of characters is absent in Chelicerata. We suggest that this character set strongly supports the Mandibulata hypothesis (Myriapoda + (Crustacea + Hexapoda)) as opposed to the Myriochelata concept (Myriapoda + Chelicerata). The evolutionary implications of our findings, particularly the plasticity of glomerular shape, are discussed. - Supported by the Max Planck Society and HA 2540/8.

Symposium 21 "Molecular and neural basis of taste detection" Wednesday 27 June

Psychophysical analysis of the contribution of the T1R2 and T1R3 proteins in taste responsiveness to sweeteners in a mouse model

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The T1R2 and T1R3 proteins form a heterodimer that binds with sweeteners and are expressed in taste receptor cells as well as other potential sugar-sensing tissues such as the gut, pancreas, and brain. Since their seminal discovery, some questions have been raised in the literature about whether the activation of the T1R2+3 heterodimer is necessary and sufficient for the generation of all sweet taste. We have been taking a behavioral approach toward this question by assessing the perceptual capacities of knockout (KO) mice lacking one or both of these proteins. In brief access taste tests, T1R2 KO and T1R3 KO mice displayed severely impaired or absent concentration-dependent licking in 5-s trials of saccharin, sucrose, glucose, maltose, and maltotriose. Interestingly, concentration-dependent responding to maltose and maltotriose, appeared with repeated testing, or, in the case of sucrose, appeared after prior experience with Polycose, a glucose polymer mixture. The experience-dependent development of such responsiveness is absent or severely impaired in double KO mice lacking both subunits. However, both single and double KO mice display relatively normal concentration-dependent licking to Polycose on the first session of testing albeit with slightly lower lick rates in the double KO mice. We recently trained T1R2 and T1R3 KO mice in an operant conditioning-based taste signal detection task and then measured psychometric detectability functions for NaCl, sucrose, Polycose, glucose, and maltose. Taste detection of the sugars was markedly impaired or absent in both KO genotypes, but was normal for NaCl and relatively normal for Polycose with some slight impairment in performance. Collectively, the findings from our studies confirm the primacy of the T1R2+3 heterodimer as the key receptor in the taste sensing of common sweeteners. At the same time, there appears to be a T1R2+3-independent mechanism that contributes to the oral sensing of Polycose. NIH R01-DC004574

Symposium 3 "Chemosensory receptors in non-chemosensory tissues" Saturday 23 June

Chemosensory Signaling, Ca^{2+} Dynamics and Diverse Behavioral Phenotypes in Human Sperm

Marc Spehr[1], Thomas Veitinger[1], Jeffrey R Riffell[2], Sophie Veitinger[1], Jaclyn M Nascimento[3], Annika Triller[4], Charlie Chandsawangbhuwana[5], Katlen Schwane[4], Andreas Geerts[6], Frank Wunder[6], Hanns Hatt[4], Michael W Berns[7], Eva M Neuhaus[8] and Richard K Zimmer[9]

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In the female reproductive tract, mammalian sperm undergo a regulated sequence of pre-fusion changes that 'prime' sperm for fertilization. Among the least understood of these complex processes are the molecular mechanisms that underlie sperm guidance by environmental chemical cues. A 'hard-wired' Ca^{2+} signaling strategy that orchestrates specific motility patterns according to given functional requirements is an emerging concept for regulation of sperm swimming behavior. The molecular players involved, the spatiotemporal characteristics of such motility-associated Ca^{2+} dynamics, and the relation between a distinct Ca^{2+} signaling pattern and a behavioral sperm phenotype, however, remain largely unclear. Previously, we identified a member of the odorant receptor family, OR1D2, as a chemosensor mediating human sperm chemotaxis. Here, we report functional characterization of two further sperm chemoreceptors. Using

complementary molecular, physiological and behavioral approaches, we comparatively describe sperm Ca^{2+} responses to specific agonists of these novel receptors and bourgeonal, a known sperm chemoattractant. We further show that individual receptor activation induces specific Ca^{2+} signaling patterns with unique spatiotemporal dynamics. These distinct Ca^{2+} dynamics are correlated to a set of stimulus-specific stereotyped behavioral responses that could play vital roles during various stages of pre-fusion sperm-egg chemical communication.

Poster session II Poster #224

Functional characterization of G-protein coupled vomeronasal receptors using a virus-based expression system

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The mouse vomeronasal organ (VNO) contains approximately 300 G-protein coupled receptors, including vomeronasal receptors type 1 and 2 (V1Rs and V2Rs) and formyl peptide receptor family (FPRs) that participate in the detection of chemosensory ligands. Matching socially relevant molecules with their specific receptors provides important knowledge about their function and biological relevance. Thus far, few receptors have been matched with specific ligands, in part, because of the unavailability of suitable heterologous expression tools. To overcome this problem, we used a Herpes simplex virus 1 (HSV1)-based ex vivo expression system for functional overexpression of vomeronasal receptors. We cloned different V2R and FPR cDNA sequences into a HSV1-based amplicon expression vector (pHSV-IRES-GFP) for bicistronic expression of the cloned receptor together with a GFP reporter. This construct is packed into virions and then used for infection of freshly dissociated primary VNO-neurons from wild type mice. We used fura-2 live cell calcium imaging on the GFP-expressing VNO infected cells to obtain a functional characterization of single vomeronasal receptors in response to specific molecules. A battery of putative ligands that includes previously identified peptide and protein pheromones was tested in HSV1-infected VNO-neurons. We observed that one member of the FPR family (FPR-rs1) when expressed in VNO-neurons can be specifically activated by classical Fpr ligands. The pharmacological profile observed in the infected cells is similar to that of Fpr-rs1 in heterologous expression. Moreover, a subpopulation of dissociated VNO-neurons in wild type mice has similar functional properties. These data indicate that our virus-based expression system can be used to identify specific vomeronasal receptor ligand interactions, contributing to understanding chemosignal-based mammalian communication.

Symposium 14 "Higher olfactory processing - Delwart Symposium" Tuesday 26 June

Specialized neurons that promote stereotyped behavior in the mouse

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Fear behavior is highly stereotyped and evolutionarily conserved. The mammalian brain regions that house fear-promoting neurons have been known for decades; however we do not know how the brain generates fear, or any other behavior. Lesion and stimulation studies have identified two mammalian brain regions important for innate fear; one responsible for generating the emotion of fear and a second that initiates fear behavior (the medial amygdala, MeA, and the ventral medial hypothalamus, VMH respectively). The MeA and the VMH are both functionally and molecularly heterogeneous and molecular markers to identify and manipulate the subset of neurons that generate fear have not been identified. The ability to 'find' the relevant neurons within these brain regions would enable first study of their function. We have previously purified a fear-promoting semiochemical. Remarkably, when an animal sniffs recombinant versions of these ligands, the fixed action pattern of fear behavior is activated. We are using this fear-promoting ligand to activate, map, and study the precise ensemble of neurons throughout the brain that generate innate fear.

Poster session II Poster #70

Interfering with local interneurons in the *Drosophila* antennal lobe: What is their contribution to the olfactory code?

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The antennal lobe (AL) in insects and the olfactory bulb (OB) in vertebrates share many similarities. Both consists of glomerular structures formed by olfactory sensory neurons (OSNs) representing the input, local interneurons (LNs) interconnecting different glomeruli and projection neurons (PNs) in insects or mitral and tufted cells in vertebrates relaying the output information to higher processing centers. The fruit fly *Drosophila melanogaster* represents a perfect model system to study olfactory processing, since available genetic tools allow interfering with single neurons within the AL. Optical imaging studies have shown that the OSN patterns as well as the PN patterns are odor specific but, however, not identical. LNs may provide the neuronal substrate of this computation. We therefore target different LN subpopulations by expressing a temperature sensitive form of *shibire* in LNs. We manipulated one subpopulation of multi-glomerular inhibitory LNs and performed calcium imaging of OSNs. Blocking synaptic transmission in these LNs via *shibire* resulted in two effects: (i) Glomeruli which were initially inhibited dropped out of the distinct odor pattern. (ii) Initially excited glomeruli decreased their activity. Both effects can be related to the blocking of this LN subpopulation since control flies were not affected. Visualizing the population response using principal component analysis showed that the block of synaptic transmission in these LNs resulted in a lost separation between odors at the glomerular level, whereas odor separation in the population response of the control flies stayed unaffected. Thus, our preliminary results support the existence of feedback from the AL network to its input represented by the measured OSNs. However, this is work in progress which we hope to extend in the nearest future by manipulating other LN subpopulations as well as performing calcium imaging at the output level (PNs) of the AL.

Poster session I Poster #71

Inhibitory projection neurons bias the evaluation of behaviorally relevant odors in the lateral horn area of *Drosophila melanogaster*

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The well investigated olfactory system of the vinegar fly *Drosophila melanogaster* enables a deep insight into neuronal coding within chemosensory networks. Projection neurons (PNs) depict the second order neurons of the olfactory system, relaying olfactory information from the primary olfactory neuropil, the antennal lobe (AL), to higher brain centers as the mushroom body calyx (MB) and the lateral horn (LH).

Investigations of uniglomerular excitatory PNs (ePNs), which target both higher brain centers, assigned a function of the MB calyx in olfactory learning processes. For the LH a role in innate olfactory behavior is assumed but could not have been shown so far.

We study inhibitory PNs (iPNs) which develop polyglomerular innervations within the AL and project to the LH exclusively. Morphological characterization of these neurons has been done using photoactivated GFP, vibratome-immunostainings with pre- and postsynaptic markers and by reconstructing 3-dimensional maps. Physiologically, the cholinergic glomerular input could be assigned via calcium imaging of presynaptic olfactory sensory neurons as well as reconstructed single iPN neurites in the AL. The iPN-output was investigated via functional imaging in the LH and analyzed using spatial independent component analysis. The pattern recognition algorithm revealed several specific odor response domains with characteristic spatial and temporal properties in the LH. Hereby a large set of ecologically relevant odors has been applied at different concentrations. The odor response domains varied according to odor concentration as well as identity. Moreover, iPN responses are clearly represented separately in the LH.

Silencing these neurons via RNAi leads to drastic changes in hedonic valences of odors: abolishing GABA expression evokes decreased attraction to attractive odors and enhanced avoidance to repulsive odors. This indicates a crucial role for iPNs in evaluating pleasantness of ecologically important odors.

Poster session II Poster #352

Salivary secretion and autonomic nervous responses induced by taste stimulation.

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It is well accepted that salivary secretion is reflexively induced by taste stimulation especially sour stimulation through the neural networks in the brainstem. Though the perceived intensity of taste sensation varies depending on their thresholds among different individuals, effectiveness of various taste stimuli on salivary secretion has been generally examined at a constant concentration of taste solution. Thus, in order to compare the effectiveness of five basic tastes on salivary secretion we used the concentration of each taste solution above the threshold which was measured before the test session in the present study. Citric acid, NaCl, sucrose, monosodium glutamate (MSG) and quinine were used for five basic taste stimuli. After the subjects put 3mL of each taste solution in their mouths and hold it for

1min, they spit it out and the weight of secreted saliva was measured. In addition, we analyzed autonomic nervous responses to taste stimuli because salivary secretion is remarkably enhanced by parasympathetic nervous activation. Autonomic nervous activities were estimated by power spectral analysis of heart rate variability during taste stimulation. Salivary secretion rate was significantly increased by all stimulation of five basic tastes compared with no stimulation or deionized water (DW). The effect of quinine was lower than other stimuli and the effect of MSG sustained even after rinsing off the solution. Regarding autonomic nervous responses, the activity of parasympathetic nerve tended to increase during MSG stimulation and decreased during quinine stimulation compared with DW, while the activity of sympathetic nerve increased during quinine stimulation. Moreover, a low but significant relationship between parasympathetic activity and salivary secretion rate was observed. These results indicate that taste stimuli except for bitterness are effective for saliva secretion and MSG may be effective for both salivation and parasympathetic activation.

Poster session I Poster #273

The relation between variation of odor-evoked positive emotion and slow breathing.

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Odor-evoked emotional change would be closely-linked to breathing. For example, smelling pleasant odor induces deep and slow breathing pattern, such as increase of tidal volume and decrease of respiration frequency. (Masaoka et al., 2005). When such respiration pattern change occurs, the activation of the limbic and paralimbic areas relating to memory, emotion and olfaction is observed (Homma & Masaoka, 2008). In this study, we investigated a possible relation between respiration pattern change and emotional variation. Basic emotional change by smelling odors is pleasantness-unpleasantness, but people can feel more various kinds of emotion from smelling odors (e.g. Chrea et al., 2008). Therefore our questions are whether every pleasant odor changes respiration pattern or there are any other factors derived from the specific emotional types which are decisive to change respiration. We used 4 odors evoking different kinds of positive emotion (activeness, calmness, impression and refreshing) and compared respiration pattern changes between these emotions.

Twenty-six healthy adults have participated to the experiment. Firstly, the participants answered their felt emotions by a questionnaire (Suzuki et al., 2003). Next, they were presented 4 emotional odors in a random order 8 times each and measured respiration parameters. After respiration measurement, the participants were asked to smell 4 emotional odors

again and evaluate emotional changes during the odors had been presented in the respiration measurement.

Compared to odorless air, all 4 odors increased tidal volume, however, an odor inducing feeling of impression showed a significant increase of tidal volume with long expiration time more than those of another odors. We suggest that odor-evoked pleasant and impressive emotions stabilize respiration pattern more than odors induced emotions of activeness, calmness and refreshing.

Poster session I Poster #313

Impact of ageing on chemosensory capacites: evidence for the existence of different patterns of alteration

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Taste and smell capacities are known to decrease with ageing. However, some authors pointed out that the variation in olfactory performance was greater among the elderly compared to the young (Koskinen & Tuorila, 2005; Laureati et al. (2008). Consequently, the aim of the present experiment was to explore the existence of possible patterns of chemosensory alteration within the elderly population. Chemosensory capabilities of elderly participants (n=60; 61-85 years) and young participants (n=63; 18-40 years) were assessed through olfactory and gustatory tests involving processes ranging from low cognitive level (detection, discrimination) to high cognitive level (categorisation, identification). All of the eight chemosensory scores used to assess the chemosensory abilities of the participants were associated with a significant age effect. However, five of these scores were associated with a greater variability in the elderly than in the young panel. According to a Hierarchical Cluster Analysis, four clusters were observed in the elderly. A first cluster (25% of the elderly panel) includes participants who performed as well as the young. A second cluster (25%) includes participants with very low gustatory scores while they scored as well as the young panel on the olfactory tests. A third cluster (43%) includes participants with very low odour discrimination score despite odour detection scores closed to the ones observed in the young population. A last cluster (7%) includes participants with a severe alteration of odour detection (close to anosmia), while they were still able to perceive salt and bitter tastes. Taken together, these results give a new insight of the impact of ageing on chemosensory perception. In particular, they highlight the fact that ageing can impact independently on olfactory and gustatory capacities, and that for a non negligible part of the elderly, chemosensory capacities are similar to those of young individuals.

Poster session II Poster #354

Sexual dimorphism in a retention index of extinction memory after conditioned taste aversion learning in mice

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After the conditioned taste aversion (CTA) learning, the extinction of CTA memory is induced by repeated presentations of the conditioned stimulus without the unconditioned stimulus such as a malaise. Recent studies have demonstrated that the extinction is a process of relearning, where mice acquire the extinction memory of CTA. When male mice (C57BL/6) in pre- or post-sexual maturation period (pre- or post-mice) underwent the conditioning period followed by the extinction period, the post-mice showed significantly higher retention index (RI) of extinction memory than pre-mice, but not in female mice. In contrast, no difference in RI of CTA memory was observed between pre- and post-mice in either sexes. These results suggest that there is the sexual dimorphism only in the RI of extinction memory. Several previous works

have indicated that the blood testosterone level transiently increases in male pre-mice. The chronic administration of testosterone or 5α-dihydrotestosterone into the castrated mice during pre-sexual maturation period resulted in an enhancement of RI of extinction memory in both sex. These results suggest that there is the enhancing effect of testosterone on the retention of the extinction memory in female as well as male mice, and the sex difference in maturation of retention mechanism of extinction memory is caused by the effect of testosterone during pre-sexual maturation period.

Poster session I Poster #355

Olfactory exposure can decrease appetite for snacks differently according to the taste types: Crossover of olfactory sensory specific satiety.

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The present study set out to determine whether olfactory exposure of vanilla (or soy-sauce) can decrease appetite for sweet (or salty) snacks. Thirty-two university students were randomly assigned to either vanilla or soy-sauce condition. The participants repeatedly smelled vanilla (or soy-sauce) for 19 times with 15 s intervals. Before and after the odor exposure, they rated the appetite for two types of sweet snacks (butter cookies and chocolate chip cookies) and two types of salty snacks (soy-sauce taste rice crisps and salt taste rice crisps) by seeing and smelling snacks. Finally, the participants were left alone after being instructed to feel free to eat as much snacks as they wanted. The results of relative change in the appetite ratings for snacks showed a significant interaction between conditions and taste types. Vanilla exposure decreased appetite for sweet snacks, and soy-sauce did for salty snacks. Detailed analysis showed that similarity of smell properties for snacks and exposed odors negatively correlated with appetite for the snacks. The amount of each snack intake varied due to olfactory exposure. Intake of butter cookies was more suppressed in vanilla group than in soy-sauce group. In this experiment, neither of the exposed odors was identical to the smell of snacks. However, vanilla odor is associated with sweet taste generally, and soy-sauce odor reminds us salty taste. Previous experience or knowledge might cause this type of effects. This report might be the first one to show the crossover of olfactory sensory specific satiety can modulate eating behavior.

Poster session I Poster #353

Identification of PTC "non-taster" Japanese macaques caused by TAS2R38 dysfunction

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Bitter taste perception is mediated by TAS2R family, and has evolved as a key defense mechanism against the ingestion of harmful and bioactive substances. It is likely that these genes evolved by means of influences of regionally specific diets during mammalian evolution. To understand the evolutional process of bitter taste recognitions, we investigated genetic polymorphisms of TAS2R38, which recognizes phenylthiocarbamide (PTC), in 409 Japanese macaques derived from 12 regions in Japan. As a result, we identified 12 single nucleotide polymorphisms, and inferred 13 haplotypes. One haplotype identified in a habitat region of Kii peninsula had a mutation in the initiation codon, suggesting that the coding protein of this haplotype is not functional. To examine the phenotype of this haplotype, we conducted behavior test and functional assay of expressed protein in cultured cells. Both of experiments revealed that the mutation at the start codon diminished the receptor sensitivities. Thus, we found PTC "non-taster" Japanese macaques in a specific region of Japan. TAS2R38 also recognizes natural ligands, glucosinolate and limonin contained in Brassica and Citrus plants, respectively, that suggests relationships with wild environments. We will discuss the relationship between the existence of region specific "non-taster" individuals and their feeding behaviors in the wild.

Poster session I Poster #109

The amphibian olfactome

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Background: The sense of smell helps animal species to evade predators, localize prey and recognize viable mates. Odors are a rich source of information, and are perceived by sophisticated olfactory systems, that have evolved over time. In humans, memoirs, thoughts, emotions, and associations are more readily reached through the sense of smell than through any other channel, suggesting that olfactory processing may differ considerably from processing in other sensory modalities. The molecular age in olfaction initiated in 1991 with the discovery of a large multigene family of olfactory receptors in rat by Linda Buck and Richard Axel (Buck and Axel, 1991).

Methods: Our study focuses on Western clawed frog (*Xenopus tropicalis*), a diploid organism that can be considered an evolutionary bridge between aquatic and terrestrial life. Several distinct differences between teleost and tetrapods olfactory receptor repertories have been reported, and a stringent analysis of the olfactory system of early and partially still aquatic tetrapods such as *Xenopus* should throw light on the evolutionary events leading to this transitions. Two olfactory receptors vomeronasal type1 and vomeronasal type 2 (V1R,V2R) receptor families of *Xenopus tropicalis* were retrieved, using homology data mining on publically available genomic databases for the vomeronasal type 1 (V1Rs) and vomeronasal type 2 (V2Rs) receptors families followed by Phylogenetic analysis. We have also begun to analyze the expression of olfactory receptors by *in situ* hybridization of tadpole olfactory epithelium.

Results: We identified 23 vomeronasal type 1 (V1Rs) and more than 500 vomeronasal type 2 (V2Rs) olfactory receptors, considerably more than previously published (Saraiva and Korsching, 2007; Ji et al, 2009) and for V2Rs the largest repertoire of any species analyzed so far.

Discussion: Working with *Xenopus tropicalis* as a model organism can help us to understand the evolutionary history of the olfactory system in vertebrates. Our analysis shows:

- *X. tropicalis* has undergone massive expansion in the V2R gene family, presumably to accommodate between water to air odor detection.

- *X. tropicalis* V1R represent the transition between the teleost and tetrapods V1R repertoire, as they underwent moderate species specific expansion.

- In comparison to fish, Xenopus have formed an additional olfactory organ called vomeronasal organ (VNO), which however houses only one of the receptor family out of two known to be expressed in mammalian VNO.

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Poster session II Poster #348

Trigeminal modulation of the olfactory system

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The sense of smell combines input from the olfactory and the trigeminal systems. The trigeminal system not only conveys somatosensory information about irritants and temperature stimuli, but it also contributes to the detection of most odourous compounds. Moreover, psychophysical and electrophysiological studies suggest that the main olfactory system and the trigeminal system influence each other´s sensory properties. This interaction may take place both on the peripheral and on the central level of information processing.

In the olfactory epithelium (OE), antibodies against the trigeminal peptides Substance P (SP) and Calcitonin Gene-Related Peptide (CGRP) labeled trigeminal fibers that seem to be associated with a heterogenous cell population at the

apical surface. The nature and function of this connection needs to be determined. CGRP inhibits odour-induced electrical potentials (EOG), hence modulating the afferent olfactory signal. Within the olfactory bulb (OB) trigeminal innervation was detected in the glomerular layer and near the mitral cells. Innervation is most prominent in the ventral region of the bulb. A recent study (Kobayakawa et al. 2007; Nature 450:503) suggested that the dorsal regions of the bulb process innate aversive odour responses, while learned responses are processed more ventrally. We examine the hypothesis that trigeminal innervation may play a role in the formation of odour memory through its impact on the ventral bulb. One important issue is the localization of the receptors for SP and CGRP. We were able to show that the receptors for both peptides are expressed in the OE and in the OB, but the exact location needs to be explored in more detail. In future, we want to study trigeminal effects on olfactory function by combining electrophysiology, expression studies, imaging techniques, and psychophysics. Our approach is designed to advance our understanding of the trigeminal role in odour detection, discrimination and memory.

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Poster session I Poster #161

Neural circuit mechanism underlying behavioral transition from attack to parenting toward pups in male mice.

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Sexually-naïve male mice show robust aggressive behavior toward pups. However, during the cohabitation period with the pregnant female after their first mating, the proportion of male mice exhibiting aggressive behavior toward pups gradually decreases. Subsequently, when these males become fathers, they turn to display parental behavior toward pups, similar to the maternal behavior by mothers. To elucidate the neural circuit mechanism underlying this behavioral transition, we examined the brain regions differently activated by pup exposure between sexually-naïve male mice and father mice, using c-Fos expression as a marker for neuronal activation. We found that, upon pup exposure, subsets of neurons in the brain regions along the vomeronasal neural pathways (accessory olfactory bulb, amygdala, and hypothalamus) were more strongly activated in sexually naïve male mice than those in fathers. Notably, c-Fos expression was downregulated in the vomeronasal organ of fathers than that of sexually naïve males upon pup exposure. Surgical ablation of the vomeronasal organ from sexually-naïve males resulted in abrogation of the aggressive behavior toward pups and simultaneously led to expression of parental behavior.

These results suggest that the chemical cues inducing aggressive behavior toward pups are received by the vomeronasal organ and activate the select vomeronasal neural pathways in sexually-naïve male mice but non in father mice. Thus the downregulation of pup pheromone-induced neural activation in the vomeronasal organ might be important for behavioral transition from attack to parenting in male mice.

Poster session I Poster #185

Molecular basis of CO_2 sensing in the mouse olfactory system

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Carbon dioxide (CO_2) is an important environmental cue for many organisms. In the mammal, mouse, rat and guinea pig are known to have a CO_2 sensor in the olfactory epithelium (OE). Mice can detect CO_2 at concentrations around the average atmospheric level by olfaction. In the ventro-lateral region of the mouse OE, there is a unique subset of olfactory

sensory neurons (OSNs), termed GC-D OSNs, which express *carbonic anhydrase 2* (*Car2*) and *guanylate cyclase-D* (*GC-D*), instead of *odorant receptor*. In GC-D neurons, Car2 and GC-D function as a sensor for CO_2 and urinary peptides, respectively. Further, it was reported that GC-D OSNs also detect carbon disulfide (CO_2) and mediates food-related social learning.

Here, we report that at least two novel subsets of OSNs, where *Car2* does not express, respond to CO_2 as well. In contrast to GC-D OSNs, these CO_2-responding neurons did react to neither urinary peptides nor CS_2. Interestingly, acidic pH solution activated only half of Car2-CO_2 sensor cells. This means that Car2- CO_2-responding OSNs can be divided into two types: the one is CO_2-sensing (type A); the other acidic pH-sensing (type B). The treatment of a carbonic anhydrase inhibitor, acetazolamide, suppressed the response to CO_2 in the type A cells. Among 16 genes encoding the carbonic anhydrase family, we have found that instead of *Car2*, *carbonic anhydrase 7* (*Car7*) is expressed in a subset of OSNs. Car7 plays a role of CO_2 sensing in the Car2$^-$ OSNs. These results suggest that mice sense CO_2 not only with GC-D OSNs, but also with the novel subsets of OSNs in the OE.

Poster session I Poster #227

The effects of intaking trigeminal stimulants on the human central nervous system

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Various stimulus modalities affect Human beings during eating process. While eating, various stimuli including 5 tastants and other gustatory and / or somatosensory sensations provide information about the food. This modal information is integrated and recognized as 'Flavors'. It is essential to understand these psychophysiological properties as it provides useful data to develop functional foods.

We investigated the effects of agents responsible of trigeminal sensations to the Human CNS and ANS using respectively Contingent Negative Variation (CNV) and skin temperature as indices. 100 ml aqueous solution of vanillyl butyl ether (VBE), l-menthol (LM) and carbonated water (CW) were used as hot, cooling and tingling stimuli respectively. The stimulant effects were evaluated by comparing the indices before and after the intake. In the CNV experiment, all 3 stimulants increased the subjects' arousal levels after the sample ingestion whereas no significant changes were observed with water. Although the arousal level was still high at 20 min. for VBE and LM, the effect of CW did not last as long. In parallel, subjects were also asked to rate the general stimulus intensity using a 9-point Likert scale during the experiment. The evaluation results for all samples, including water showed a maximum intensity just after intake, then decreased over time nevertheless the intensity remained significantly strong for until 20 min. for LM and VBE. This indicates that the subjective stimulus intensity at a certain point can impact the arousal level. The skin temperature of nose and fingertip decreased immediately after the subjects put VBE sample in their mouth. This suggests the first impact of VBE result in activating the subjects' sympathetic nervous system (SNS). Our findings revealed that intaking of 3 reported agents can affect both Human CNS and ANS. Particularly, CNV and skin temperature are good method to measure duration and impact of each stimulus, and other properties.

Poster session II Poster #228

Analysis of a novel protein found in olfactory sensory neuron cilia

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Olfactory sensory neuron (OSN) cilia are specialized for encoding and transducing odor information. Although many of the signal transduction components are known, there may be other proteins that modulate olfactory responses. Additionally, many components necessary for OSN cilial development and maintenance are unknown. We recently identified a novel protein, Q8BH53, that is highly expressed in murine OSN cilia and is conserved among ciliated

eukaryotes. Q8BH53 is an ARM-repeat domain-containing protein whose function is unknown. Q8BH53 is a truly unique protein; no other paralogs exist in the mouse genome. It is enriched in cilial membrane preparations of OSNs and transcripts are abundant in the mouse olfactory epithelium. Q8BH53 localizes specifically to OSN cilia but is largely excluded from the respiratory epithelium cilia that are directly adjacent to the olfactory epithelium. In addition to OSNs, *q8bh53* transcripts are also found in several other ciliated tissues, including the testes and kidney. Further functional analysis using molecular genetics will reveal the role of Q8BH53 in the olfactory system.

Poster session I Poster #229

A suppressing mechanism of the recurrent inhibition by Group II metabotropic glutamate receptors activated by endogenous glutamate release from mitral cells in the mouse accessory olfactory bulb

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The goal of our research is to understand the mechanism of synaptic transmission in the AOB that has been demonstrated to be critical to memory formation for male mouse pheromones. In the previous study, we measured the reciprocal synaptic currents from mitral cells in the AOB evoked by applying a depolarizing voltage step from –70 to 0 mV to them. e have demonstrated that an agonist for group II metabotropic glutamate receptors (mGluR2/mGluR3), DCG-IV, suppressed dendrodendritic inhibition (DDI) whereas the mGluR2/mGluR3 antagonist LY341495 enhanced it. The effects of these drugs were markedly impaired by genetic ablation of mGluR2, indicating that DCG-IV-mediated suppression of DDI is mediated by mGluR2.

In the present study, to see whether mGluR2 has similar effects on the DDI elicited by relatively more physiological stimuli (spike trains, for instance), mitral cells in slice preparations were stimulated with a threshold current stimulus of variable intensity adjusted to elicit action potential(s). IPSPs after the current stimulus were recorded using the patch-clamp technique in whole-cell configuration. AOB slices were prepared from 23- to 36-day-old Balb/c mice. In the presence of LY341495 in the bath solution IPSPs were enhanced. Together with previous results, the present results suggest that mGluR2 can be activated by endogenous glutamate release from mitral cells, which results in the suppression of the synaptic transmission from mitral to granule cells in the AOB. Our previous study has also demonstrated that DCG-IV reduced the frequency of mEPSCs recorded from granule cells with slight decrease in the amplitudes, suggesting that the suppression of this synaptic transmission by mGluR2 occur through both presynaptic and postsynaptic mechanisms.

Poster session I Poster #389

Down-regulation of sweet receptor mRNAs in diabetic mice

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[Introduction] Sweet receptors belong to the T1R (Tas1R) family and are presently identified as Tas1R1, Tas1R2 and Tas1R3. Two of these molecules form heterodimers as follows: Heterodimer of T1R1 and T1R3 to detect umami (savoriness); and Heterodimer of Tas1R2 and Tas1R3 to detect sweetness. In the present study, we compared expressions of these receptor proteins and their corresponding mRNAs, *Tas1r1, Tas1r 2 and Tas1r 3*, in diabetic and control mice in order to investigate their possible involvement in diabetes mellitus. **[Materials and methods]** Type II diabetic mice ($Lepr^{db}/Lepr^{db}$) and their control mice ($Lepr^{db}/+$), ten males in each group, were used. Diabetes was confirmed by urinary tests. Their brains were removed, fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), embedded in paraffin, and processed for immunohistochemistry. In addition, the mRNAs, *Tas1r1, 2 and 3*, were examined by in situ hybridization, reverse transcription PCR (RT-PCR), and quantitative real-time PCR (qPCR). **[Results]** Immunohistochemistry revealed that all three T1Rs were localized in the soma of neurons in both groups. The localizations of mRNAs of *Tas1r*s by in situ

hybridization were essentially the same as those by immunohistochemistry. RT-PCR revealed positive bands for all three *Tas1r* mRNAs in both groups. On the other hand, qPCR revealed that the amount of *Tas1r2* in diabetic mice was about a half of that of control mice, although amounts of *Tas1r1 and Tas1r3* remained almost the same across groups. **[Discussion]** The present study demonstrates that T1R family members are expressed in neurons functioning as chemical sensory cells for umami and sweetness. The present findings of the qPCR showing a smaller expression of Tas1r2 in diabetic mice suggest that down-regulation of sweet receptor molecules might occur only in diabetic mice and not in control mice, since the neurons in the diabetic mice have been exposed to high glucose level for weeks.

Poster session II Poster #230

High-fat diet causes loss of olfactory sensory neurons and their projections while concomitantly impairing olfactory learning and discrimination in mice

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Previously we have demonstrated modulation of mitral cell biophysical properties due to acute or *in vivo* stimulation by insulin or glucose, or chronic diet-induced obesity (DIO). Here we explored the effect of a fat-enriched diet on neuroanatomy and behavior using mice with a gene-targeted deletion of the *Shaker* channel, Kv1.3-/-, which results in a "Super-smeller" phenotype and a resistance to DIO and associated hyperglycemia. Wild-type (WT) and Kv1.3-/- mice were maintained on control chow (CF, 13% fat), moderately-high fat diet (MHF, 32% fat), or a high-fat diet (HF, 60% fat) for 6 months. A significant loss of M72-expressing olfactory sensory neurons (OSNs) as well as total mature OMP+ OSNs was observed in all MHF-diet mice. The size of the M72 glomerulus was reduced with the concomitant decrease in the OSN axonal projection. Higher-fat diets also simultaneously increased the number of apoptotic OSNs and proliferative basal cells, suggesting a disruption of olfactory epithelium regeneration. In order to test olfactory performance, mice were challenged in a Knosys olfactometer for go/no-go operant conditioning involving discrimination trials between a conditioned (S+) and an unconditioned odorant (S-). Higher-fat challenged mice, regardless of genotype, required twice the time to achieve a minimum 80% accuracy required to continue in the S+/S- discrimination task. HF-challenged WT mice had a reduced accuracy to recognize S+, difficulty in discriminating at asymptotic levels of performance between two dissimilar odors, and only one could relearn a task following switch of the S+ with the S-. Interestingly, the obesity resistant Kv1.3-/- mice maintained on a HF diet, had increased olfactory discrimination ability over CF diet, but were slower to relearn following a S+/S- switch. Our data demonstrate that a deregulation of energetic metabolism, independent of weight gain, can significantly impair the function and structure of the olfactory system.

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Poster session II Poster #98

A large-scale behavioral screen for neurons responsible for sugar response in *Drosophila*

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The ability to sense gustatory stimuli and distinguish palatable from toxic substances is important for the survival of all animals. While neurons that are involved in driving and modulating appetitive behavior have been described, comprehensive characterization of the neuronal networks that are important for such behavioral responses remains elusive. To this end, we investigated behavioral responses of many transgenic Drosophila lines to sucrose. In these flies, subsets of neurons can be conditionally blocked. Therefore, the importance of these neurons can be assessed with defective sucrose responses. We show the results of the screen and the approach to identify required neurons.

Poster session I Poster #381

Changes in gustatory and olfactory perceptions of patients with Major Depression treated with Vagus Nerve Stimulation (VNS)

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Backround: Inspite of the importance of life quality during the psychiatric treatment the effetcs of VNS in patients suffering from Major Depression remain to be determind. Thus we investigated olfactory and gustatory functions before and during vagus nerve stimulation (VNS) in a group of 9 patients with therapy-resistant depression, implanted with a VNS system.

Patients and Methods: Gustatory and olfactory functioning were tested using the Sniffin'-Sticks Test for olfactory threshold, discrimination, identification, hedonic and intensity perception and the one drop test for gustatory threshold and intensity perception. Subjects participated in two sessions with the vagal stimulator switched on and off, respectively. Results: Under conditions of stimulation of the VNS, there were statistically significant differences in the thresholds of perception for sweet, sour and bitter. A statistical trend could be observed for the threshold of salty. Patients perceived the stimulus intensity of sweet significantly higher during the on mode compared to the off condition. A similar effect could be observed for the intensity estimates of sour, but this observation reached a statistical trend only. There were no statistically relevant differences concerning olfactory perception.

Conclusion: Our study clearly showed that gustatory perception in patietns with Major Depression can be modified by vagal nerve stimulation. The detectable effect of Vagal Nerve Stimulation on specific regions of the brainstem as the Nucleus tractus solitarii rises the question to what extend gustatory perceptions are modifiable by vagal stimulation. The impact of direct vagal stimulation may go far beyond the usual approach of an immediate effect of vagal afferent projections to the Locus coeruleus. Especially on the basis of long-term studies neuronal adaptations in a sense of changes in neural plasticity have been postulated as a main mechanism of VNS.

Poster session II Poster #176

The potential for long-term individual recognition in the anal gland secretion of Eurasian beavers (*Castor fiber*)

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The social life of mammals depends strongly on chemical signals because they reveal detailed information about the sender including sex, age, social status, physical and reproductive condition, and individuality. Although an individual's scent will be subject to changes throughout life, allowing individual recognition over time is beneficial, especially when individuals have complex social interactions or are likely to meet on several occasions.The existence of individual-specific odours has been established in a wide variety of mammals. However, long-term studies of mammals are rare, and few studies have investigated how individuals' chemical profiles remain recognizable over a period of many years. In this study we used gas chromatography – mass spectrometry to investigate the chemical profile of anal gland secretion (AGS) samples from Eurasian beavers (*Castor fiber*)collected between 1997 and 2009. We hypothesised that the AGS profiles would provide multiple messages without losing the individual-specific profile. The AGS profile of each individual did change over time, but scent profiles from same individuals were still significantly more similar to each other than to scent profiles from different individuals. Thus, our results indicate that the AGS of the Eurasian beaver carries the potential for long-term individual recognition.

Poster session II Poster #410

Establishment of a new cell-based assay to measure the sweetness intensities of ligands including fluorescent substances

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Taste receptors have been defined at molecular level in the past decade, and cell-based assays have been developed using cultured cells heterologously expressing these receptors. The most popular approach to detecting the cellular response to a tastant is to measure changes in intracellular Ca2+ concentration using Ca2+-sensitive fluorescent dyes. However, this method cannot be applied to food-derived samples that contain fluorescent substances. In considering the industrial importance of taste-modulating compounds of food origin, establishing a high-throughput screening system that is useful to evaluate fluorescent food-derived materials will no doubt be necessary. To establish an assay system that would be applicable to fluorescent samples, we tested the use of jellyfish-derived Ca2+-sensitive photoproteins as Ca2+ indicators in a human sweet taste receptor assay. Four types of photoproteins, i.e., aequorin, obelin, clytin and clytin-II, were transiently transfected into cells that stably coexpressed the human sweet taste receptor, hT1R2/hT1R3, together with a functional chimeric G-protein, hGα16gust44, and the cellular responses to sweetener were measured using a luminescence microplate reader. By comparing the luminescence intensities and signal/background ratios obtained using each photoprotein, we selected the most suitable photoprotein for the taste receptor assay. Using these systems, we successfully detected receptor activation in response to sweetener, even when fluorescent compounds coexisted. This luminescence-based assay will be a powerful tool to objectively evaluate the sweetness of food-derived samples even at industry level.

Poster session I Poster #411

A common mouse strain lacks sweet, umami, bitter and calcium taste due to a mutation in Itpr3, the inositol 1,4,5-triphosphate receptor type 3

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The BTBR mouse strain originated ~60 years ago and is used commonly in immunological and developmental studies. In 48-h two-bottle choice tests, BTBR mice were indifferent to low and moderate concentrations of saccharin, sucrose, inosine monophosphate, denatonium benzoate, quinine hydrochloride, $CaCl_2$ and calcium lactate. To discover the gene(s) responsible, we crossed BTBR T^+ *tf*/J mice with NZW/LacJ mice, produced 610 F^2 hybrids, and measured their preferences for representative taste compounds. A genome scan with 626 informative markers revealed a QTL on Chr 17 with strong linkage to saccharin preference (LOD = 100.8) and calcium intake (LOD, $CaCl_2$ intake = 56.7; calcium lactate intake = 58.6). We isolated this region in a congenic line by marker-assisted serial backcrossing. After 12 generations, the phenotypes were supported by a 0.8-Mb introgressed region on Chr 17 bounded by recombinations distal to *rs47196150* (26.66 Mb) and proximal to *rs3656446* (27.48 Mb). This congenic interval contains 21 known and predicted genes, including *Itpr3,* the inositol 1,4,5-triphosphate receptor type 3 gene. The protein product of *Itpr3*, ITPR3, is a component of the GPCR-mediated taste transduction cascade, and mice with knockout of *Itpr3* cannot detect sweet or bitter tastes. We have recently discovered a novel deletion in exon 23 of *Itpr3* of the BTBR strain. We conclude that a spontaneous mutation in the *Itpr3* gene of the BTBR strain has rendered it unable to detect GPCR-mediated tastes, including several sweet, umami, bitter, and calcium taste compounds. The BTBR strain is a widely available and inexpensive model for studying GPCR-mediated taste loss.

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Symposium 19 "Preference for umami taste controlled by chemical senses - Ajinomoto Symposium" Tuesday 26 June

Physiological significances on glutamate signaling as umami taste, visceral information and brain functional changes due to homeostatic control after mealing

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Gustatory and visceral stimulation of food regulates digestion and absorbed nutrient utilization. Free glutamate (Glu) in foods induces the umami taste sensation that increases food palatability and promotes digestion. Dietary glutamate is also the main source of energy for the intestinal mucosal absorption and metabolism, thus, only a trace amount of Glu reaches the general circulation even after the intake of dietary protein and Glu added in foods when umami taste sensation is not sufficient to be palatable. In addition to these physiological roles, we demonstrated a unique gastric sensing system for Glu. Glu is the only amino acid that activates rat gastric vagal afferents from the luminal side possibly via metabotropic Glu receptors on mucosal cells. Functional MRI (4.7T) analysis revealed that luminal sensing with 1% (w/v) Glu (most preferred concentration) in rat stomach activates the medial preoptic area (body temperature control) and the dorsomedial hypothalamus (basic metabolic regulator), resulting in diet-induced thermogenesis without changes in food intake. Interestingly, rats fed a high fat and high sugar diet with free access to 1% Glu and water showed the strong preference for Glu solution, and subsequently lower fat deposition, weight gain and blood leptin compared with those without Glu. In addition, these brain functional changes were abolished in the case of total vagotomized rats. Total and partial gastric and celiac branches vagotomy induced similar results to each other, suggesting that glutamate signaling should be yielded from the luminal side of the alimentary tract to contribute to the maintenance of our healthy dietary life.

Symposium 3 "Chemosensory receptors in non-chemosensory tissues" Saturday 23 June

Searching an endogenous natural ligand for an olfactory receptor expressed in non-olfactory tissues

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In mammals, detection of numerous volatile signals in the external world is mediated by several hundred odorant receptors (ORs) expressed in olfactory sensory neurons. In the past several years, heterologous assay systems for ORs have been developed, and many ORs have been paired with cognate odorants by screening compounds that are commercially available or can be obtained from fragrance companies. However, the ligands for ORs in a natural environment are distinct from odorants available in a laboratory, but are odorants derived from food or enemy, or from urine, feces, or various secretions of other individuals or species. In addition, ORs are also expressed in non-olfactory tissues such as testis, muscle, developing heart, brain, and spleen, and presumably these ORs are sensing small metabolic compounds for specific biological functions. Thus, natural ligands received by ORs expressed in a nose or in non-olfactory tissues are largely unknown. The biggest problem that has hampered screening natural ligands for ORs is a high background response to crude tissue extracts in a calcium or cAMP assay in a heterologous cell. We herein developed a highly efficient OR assay with no background that could be utilized to purify endogenous natural ligands for ORs, starting from crude extract samples. To determine the usefulness of our strategy, we first searched natural ligands of mouse ORs secreted from several exocrine gland extracts. By OR assay-guided fractionation and chemical analysis, we identified (*Z*)-5-tetradecen-1-ol as a novel natural OR ligand that is bio-synthesized in the male preputial gland and then secreted into male urine. We next attempted to identify an endogenous natural ligand for ORs expressed in various tissues, and the progress will be presented.

Poster session II Poster #412

Norepinephrine and NPY modulate gustatory input to pre-oromotor neurons

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Visceral and gustatory signals have the capacity to increase or decrease food intake via circuits complete within the brainstem. These circuits consist of the relevant afferent pathways, interneurons, and oromotor neurons effecting the consummatory response. Visceral afferents signaling satiety or glucoprivation are thought to include catecholamine (CA) neurons in the caudal nucleus of the solitary tract (cNST) and ventral medulla, a significant proportion of which co-localize neuropeptide Y (NPY). Interneurons projecting to the oromotor nuclei are located in the brainstem reticular formation; however it is not known if these neurons are influenced by visceral afferent signals. To determine if CA and or NPY influence pre-oromotor neurons, we recorded from identified pre-hypoglossal neurons (pre-mXII) in a brainstem slice preparation and determined if bath application of norepinephrine (NE), NPY and specific NPY receptor agonists modulated excitatory input originating from the rostral (gustatory) nucleus of the solitary tract (rNST). In most pre-mXII neurons, NE suppressed input from the rNST. NPY and specific Y1 and Y2 agonists likewise had a predominantly suppressive effect. A paired-pulse ratio analysis indicated that inhibition via Y1 was both pre- and post-synaptic compared to Y2 which was pre-synaptic. We further determined that stimulation of the cNST could drive pre-mXII neurons. Although we have not determined if these projections are CA, we did observe that alpha-2 agonists, in particular, had a suppressive effect on cNST evoked responses in pre-mXII neurons. In a separate set of studies, we infused NE into the awake behaving rat to observe its effect on taste-evoked behavior. Preliminary data suggest a suppressive effect of NE on the rate of licking. These data support the hypothesis that CA/NPY neurons in the cNST involved in satiety can suppress ingestion by modulating excitatory gustatory influences at the level of the premotor neuron.

Symposium 2 "Coding of taste across mammals: from the tongue to the cortex" Saturday 23 June

Gustatory processing in the rodent brainstem: coding and modulation

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The rodent brainstem houses 2 complex, taste processing circuits: the rostral nucleus of the solitary tract (rNST) and parabrachial nucleus (PBN). The extrinsic connections and response properties of these nuclei have been characterized but less is known of their intrinsic organization and local modulatory capacity. Our *in vivo* studies stress orderly afferent convergence that maintains notable chemospecificity in rNST neurons, a rough orotopy, and a medial to lateral organization of taste vs somatosensory function. There is dense immunostaining for P2X2 in central endings of 1^0 afferents which is restricted medially implying a selective central purinergic influence on rNST taste processing. Other modulatory rNST circuitry includes a significant population of GABAergic neurons expressing GAD67. *In vitro* data demonstrate solitary tract-evoked monosynaptic responses in some GAD67 neurons, indicating feed-forward inhibition. Different rNST efferents project to the caudal, visceral NST (cNST), reticular formation, and the PBN, regions differentially involved in reflex function and ascending forebrain pathways. GAD67 rNST neurons are distributed widely to provide a substrate for inhibitory influences on all these efferent targets. Other modulatory sources include inputs from cNST and endomorphin, enkephalin, and catecholamine fibers that distribute preferentially to reflex-related rNST regions. Multiple types of convergence in PBN arise from rNST, including inputs from taste neurons with different receptive fields. Nevertheless, many PBN neurons maintain significant chemospecificity though others are more broadly tuned especially during the phasic response. Moreover, there is dramatic overlap between NST afferents from the gustatory and visceral zones which is most prominent in subnuclei that preferentially target ventral forebrain vs thalamocortical circuits. Thus, in both NST and PBN, multiple, local modulatory substrates influence distinct neuronal subpopulations.

Poster session I Poster #119

In silico approach to identify odor metric using odor descriptions

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The first step of odor detection and discrimination of myriads of structurally diverse odorants depends on their interactions with olfactory receptors[1], whereas the perception of odors quality results from a combinatorial coding[3], whose deciphering remains now a major challenge.

Haddad *et al.* pointed out the lack of odor metrics, *i.e.* rules describing olfactory perceptual space, as an obstacle to understand olfactory coding, and proposed two physicochemical metrics[2]. More recently, a descriptive analysis was performed of the FlavorBase 2010 (FB2010; http://www.leffingwell.com), putting forward that the odor description can be successfully analyzed using a metric approach[4].

FB2010 is one of the largest collections of natural and synthetic odorant molecules (4184 entries), whose flavor and odor descriptions are based on bibliographic documents.

We performed a computational analysis using odorant descriptions of the molecules present in FB2010. First, we identified 740 odorant descriptors, 200 of them are present in at least 10 molecules. These 200 descriptors have been used to create a matrix containing the 4184 odorant molecules. According to previous studies, each element of the matrix was converted into binary values[5,6].

Four statistical analysis approaches based on odor descriptions of the molecules were applied: classical multidimensional scaling, correspondence analysis of odor descriptors, hierarchical clustering and Kohonen Self-Organizing Maps.

The obtained results highlight subsets of molecules sharing close odor descriptors, suggesting an interesting and promising way of using computational approaches to help to decipher olfactory coding.

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Poster session II Poster #72

Neuroethological analysis reveals localization of pheromone-plant odor interaction in the moth antennal lobes

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An outstanding challenge in olfactory neurobiology is to reveal the mechanisms that underline the discrimination of behaviourally relevant odours, including the mechanisms regulating the interaction between social and environmental cues. We have studied how binary blends of the main sex pheromone and host-plant cues affect odor processing and ensuing behavior in a worldwide insect pest, the codling moth *Cydia pomonella*. We show that the presence of ecologically relevant plant volatiles increases the attraction of males to a threshold dose of codlemone: in wind tunnel bioassays, a higher proportion of moths made close upwind flights and contact with the source of blends of pheromone

and plant volatiles than to the single compounds alone. Through an integrated analysis, we demonstrate that the high level of behavioral interaction between sex pheromone and host compounds is mirrored at the neurophysiological level. Calcium imaging of the primary olfactory centre, the antennal lobe (AL) showed that the presence of plant volatiles enhanced the response to a sub-threshold dose of codlemone in the Cu, the largest glomerulus of the macroglomerular complex (MGC), while suppressive interactions were observed in other parts of the AL. Intracellular recordings from AL projection neurons confirmed that synergistic responses were confined to the Cu and other glomeruli in the MGC. Our physiological analysis demonstrates that the coding of odor signals from conspecifics and plants is highly integrated in the central nervous system. This highlights the role of host plant cues in premating sexual communication and underscores that, in nature, sex signals and habitat cues are always perceived as an ensemble.

Contributed talks I "Modulation of the olfactory system (Linnaeus Symposium)" Monday 25 June

Sensory input regulates the dendritic development of specific neuronal subtypes in the mouse olfactory bulb

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Sensory input has been shown to regulate development in a variety of species and in various structures, including the retina, cortex and olfactory bulb (OB). Within the mammalian OB specifically, the development of dendrites in mitral/tufted cells is well known to be odor-evoked activity-dependent. However, little is known about the developmental role of sensory input in the other major OB population of the GABAgenic interneurons, such as granule cells and periglomerular cells. Here, we identified, with DNA microarray and in situ hybridization screenings, a glycoprotein gene 5T4 and a transcription factor gene Npas4, whose expression in the OB interneurons are dependent on sensory input. 5T4 is a transmembrane protein, whose extracellular domain contains seven leucine-rich repeats, and a short cytoplasmic domain. 5T4 overexpression in the newborn OB granule cells facilitated their dendritic branching even under the sensory input-deprived condition. By contrast, both 5T4 knockdown with RNAi and 5T4 knockout with mice resulted in a significant reduction in the dendritic branching of OB granule cells. Further, we identified the amino-acid sequence in the 5T4 cytoplasmic domain that is necessary and sufficient for the sensory input-dependent dendritic shaping of specific neuronal subtypes in the OB. Npas4 is a neuronal Per–Arnt–Sim (PAS) domain protein 4, whose N-terminal region contains a basic helix–loop–helix domain for DNA binding and two PAS domains involving in the adaptation of cellular stresses and environmental factors. Npas4 overexpression in the newborn OB granule cells facilitated their dendritic spine formation, while its translational fusion with the engrailed repressor domain showed a significant reduction of the dendritic spine formation. Thus, these results demonstrate that 5T4 and Npas4 contribute to regulate the activity-dependent dendritic development of interneurons and the formation of functional neural circuitry in the OB.

Poster session I Poster #315

Perception of pungent, taste and odor stimuli in the patients with congenital insensitivity to pain with anhidrosis (CIPA)

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CIPA is a severe autosomal recessive disorder caused by mutations in the *NTRK1* gene coding for the tyrosine kinase receptor A. Since this disease is characterized by loss of pain sensitivity and peripheral unmyelinated and small myelinated nerve fibers, it raises the possibility that oral sensation such as burning sensation and taste may be affected. In addition, ability of smell could be affected because some of the patients have mental retardation. Thus, we investigated

the sensitivities of CIPA patients to stimuli of capsaicin, tastes and odors. The sensitivity to capsaicin measured by topical application of capsaicin solution on tongue surface using small filter paper disc and the sensitivities to five basic tastes were measured by whole-mouth method. The identification capability for eight odors was evaluated using the odor stick identification test. Though all of ten patients could feel burning sensation of capsaicin, the mean threshold of the patients was remarkably higher compared with that of healthy subjects. Five patients out of eight could recognize all taste qualities showing that the patients generally possess the ability to taste foods. The mean thresholds of the five patients for sour and umami tastes were, however, significantly higher than those of healthy subjects and the threshold for bitter taste also tends to be higher. In contrast, all of seven patients could identify well the tested odors except one they had never experienced.

In conclusion, these results suggest that CIPA patients partly retain the sensitivity to chemical nociceptive stimulus such as capsaicin which is ever quite low, while their abilities to taste and smell are not basically impaired by this defect indicating that they may not have much problems in their dietary life.

Poster session II Poster #146

Effect of collapsin response mediator protein 4 (CRMP4) knockout on the olfactory bulb

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The collapsin response mediator protein (CRMP) family consists of five phosphoproteins (CRMP1-5) whose involvement is suggested in growth cone collapse and axon guidance. However, limited information is available not only on the localization of CRMP4 but also on its function. In our previous study where spatiotemporal expression changes of *crmp4* mRNA were investigated in the developing mouse brain, expression patterns of *crmp4* were classified into the following three types: (1) In most brain areas, signals were strongly detected on postnatal day 0 (PD0=birth day) or PD7, became weak or disappeared on PD14 and were not found in adults; (2) In the adult neurogenetic regions, the dentate gyrus and subventricular zone of the olfactory bulb (OB), the signals were first detected on PD0 or PD7 and remained positive thereafter; (3) In the glomerular and mitral cell layers (MCL) of the OB, the intensity of signals was the strongest on PD0 or PD7 and decreased gradually toward adulthood, but signals were still detectable in adults. These results suggest a specific role of CRMP4 in the OB, in addition to its roles in immature neurons. Hence, in the present study, we aimed to elucidate the roles of CRMP4 in the OB by using *crmp4*-knockout (KO) mice. For this purpose, we first investigated morphological changes in the OB of *crmp4*-KO mice, using DiI tracing and Nissl staining.

In the OB of *crmp4*-KO mice on PD0, MCL was significantly thinner than that in wild-type mice. The number of mitral cells in *crmp4*-KO mice was also significantly less than that in wild-type mice on PD0. These differences disappeared on PD21. Mitral cells visualized with DiI revealed that their apical dendrites became longer in *crmp4*-KO mice than those in wild-type mice. These results implied that CRMP4 plays a specific role in the development of the MCL of the OB. We are now studying olfactory disorders in *crmp4*-KO pups by measuring ultrasonic vocalization emitted in the presence of an odor stimulus.

Poster session I Poster #417

Odorant binding protein biosensors for detecting volatile organic compounds (VOCs) in vapour phase.

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Since the discovery of Odorant binding proteins (OBPs) in insects and vertebrates, their physiological role in the chemical perception of the olfactory stimuli has been widely studied. They bind chemical compounds reversibly as demonstrated for years, using ligand binding assays in liquid media. We have investigated this property in vapour phase, in

order to realise a biosensor for the detection of label- free VOCs. OBPs immobilised on different transducers can be used as sensitive layer of sensors for agro-food industries. Their selectivity toward volatile compounds related to the quality of food or pheromones allows applications in the field of logistics for the monitoring of perishable goods during transport and storage, and also as instruments for the control of useful insects and pests. Different kind of transducers can be employed for recording the responses of OBPs toward volatiles chemicals. Mass transducers such as quartz crystal microbalances were used in preliminary studies. Such techniques were then implemented on flexible substrate including interdigitated electrodes in order to assess the biochemical and electric properties of OBPs in response to the tested analytes. OBPs were immobilised on the surface of the transducer using two different methods. Covalent immobilisation by self assembled monolayer of alkanethiols on gold, or by entrapped in a sol-gel. The efficiency of the two immobilisation methods was investigated by comparing the sensitivity of the biosensors toward different concentration of target analytes. Each OBP, belonging to different species, showed a good selectivity against a specific compound; with a sensitivity of the order of parts per billion for those with higher affinity. We report here the possibility to use OBPs as the active layer of a biosensor with real time applications; allowing continuous, rapid and direct monitoring of target compounds in vapour phase.

Poster session II Poster #116

Antennular morphology in *Coenobita* terrestrial hermit crabs

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Behavioral and morphological studies on terrestrial hermit crabs (Coenobitidae, Anomura) have provided evidence that these animals have a good sense of aerial olfaction (Stensmyr et al., 2005; Harzsch, Hansson, 2008). Detection of odour molecules takes place on the lateral surface of antennules, which bears many rows of presumably olfactory sensillum-like structures, named aesthetascs. We studied the ultrastructure of antennules in different species of *Coenobita* using scanning and transmission electron, as well as confocal laser scanning microscopy. Aesthetascs are short and blunt compared to aquatic species, and form a dense field that is surrounded by a row of mechano- and/or gustatory sensilla. On the very tip of the antennulae, these sensilla are longer than the aesthetascs and form branches. Aesthetascs are surrounded by a layer of cuticle, which does not seem to have any pores. Olfactory sensory neuron cell bodies are organized in spindle-like complexes of around 300 each with branched dendrites extending to the very tip of the aesthetasc. The aesthetascs are immersed in a layer of mucous, which is most likely produced by the large glandular complex below and behind the sensory neuron cell bodies. Cuticle-lined ducts project from these glands to the cuticular surface between the aesthetascs. Pores, likely to exude the mucus, are found between the aesthetascs. The organization of the *Coenobita* peripheral olfactory system provides an interesting insight into how an olfactory system has adapted to smelling in terrestrial environments. From an evolutionary perspective, this development has occurred over a comparatively short time. In comparison with the insect olfactory system we see both similarities and clear differences.
This project was funded by the Max Planck Society

Symposium 18 "Olfactory neuroethology" Tuesday 26 June

Cnga4 channel gene knockout alters odor detection and odor discrimination of group-housed mice in a fully automated olfactometer

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In order to increase the throughput of odor discrimination testing of genetically modified mice we designed 3 fully automated olfactometers allowing simultaneous training of up to 3 groups of 10 mice 24 hours 7 days per week. Mice acquired the discrimination task as quickly as individually trained mice and reached equally high performance values. As expected, mice were highly active during night time, resulting in a substantial increase

in the number of trials per day (713 , SD 30). Discrimination time differences between simple odors and their binary mixtures reported previously (Abraham et al 2004) could be reproduced in our group training setting. Using a novel mouse strain with a Cnga4 gene knockout, we tested the importance of Cnga4 for odor discrimination and odor detection. Odor discrimination performance did not vary significantly between genotypes; however odor detection thresholds and susceptibility to background odors were increased in Cnga4 knockout mice. This confirms published results from testing mice with a Cnga4 knockout individually (Kelliher et al 2003). We demonstrate that fully automated testing of olfactory performance can be carried out without water deprivation, without isolation of mice in individual cages, and during the hours of highest activity of diurnal animals.

Symposium 5 "Interspecific chemointeractions" Sunday 24 June

Plant-mediated tritrophic interactions in the rhizosphere

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We have been studying the role of herbivore-induced plant volatiles in the attraction of natural enemies of herbivores, for instance parasitic wasps. Using maize as our model plant, we have found that these tritrophic interactions also occur belowground, where roots damaged by corn rootworm release (*E*)-β-caryophyllene, a potent attractant of entomophatogenic nematodes (EPN). Interestingly, most North American maize varieties have lost the ability to release the attractant, leading to dramatically lower nematode infection rates of pest larvae in the field. We used genetic transformation to restore the signal in an American line and in field trials we found that this transformation drastically improved the plant's ability to attract the beneficial EPN.

Our reports on the attraction of EPN towards root-produced signals have been met with some skepticism. One reason for this is that (*E*)-β-caryophyllene dissolves very poorly in water, which was the expected mode of diffusion for belowground signals. With a series of diffusion experiments, we showed that (*E*)-β-caryophyllene is one the best diffusing compounds produced by maize plants and that it diffuses in the gaseous phase rather than the liquid phase. Another reason for skepticism is the fact that previous studies implicated host-derived chemicals in the attraction of the EPN. Carbon dioxide (CO_2) in particular has been reported as an important short-range cue. We recently compared attraction of an EPN species to CO_2 and two typical inducible root volatiles and found that a combination of the ubiquitous gas and a more specific root volatile is considerably more attractive than one of the two alone. Hence, future studies on EPN foraging behavior should take into account that CO_2 and plant volatiles may work in synergy as attractants for EPNs. In my presentation I will further elaborate on our current efforts to enhance these interactions to better protect maize roots against rootworms.

Poster session II Poster #356

Both amplitude and latency of human cortical gustatory response contribute to coding of taste quality and intensity

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The spiking rate of neurons in the primary gustatory cortex has been shown to increase as a function of the concentration of tastants delivered in the oral cavity of non-human primates. In humans, metabolic neuroimaging studies have also reported dose-dependent responses in the same regions with modulations of the activation level in anterior insula and frontal opercular cortex. A similar effect on the amplitude of human cortical responses has been observed with neuromagnetic recordings. Yet, no such effect has been observed on the latencies of these responses to tastants, despite the millisecond-temporal resolution of this technique. Here, we combined electroencephalography with a gustometer to deliver tastant puffs and record cortical responses of 20 participants to taste solutions varying in quality and intensity. Results show a dose-response effect around 200 ms post-stimulation to two different concentrations of salt. Latencies are shorter and amplitudes are higher for high concentrations of salt compared to low ones (respectively 0.69M and 0.13M). These electrophysiological results mirror behavioural data showing shorter reaction times and higher intensity scores for high compared to low salt concentrations. Moreover, the combination of certain different tastants can also shorten the latency and increase the amplitude of the responses compared to the responses for the same tastants alone. Taken together, the results provide a demonstration of a dose-response effect for timing and amplitude of human brain responses most likely originating from primary gustatory areas. They also offer an electrophysiological description of interaction mechanisms between tastants in humans.

Poster session I Poster #231

Some odorant receptors tend to be susceptible to internalization after disposition to the cell membrane

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High-sensitive and selective chemical sensor is strongly required for investigation of narcotics crime, landmine detection, drug screening, and so on. Conventional semiconductor-based chemical sensors could not distinguish detailed structures of chemicals. We have tried to develop a cell-based chemical sensor, “artificial olfactory cell”, using the olfactory receptors. To build the “artificial olfactory cell” efficiently, we have to express the olfactory receptors at the cell membrane. Recent report suggested that the receptor transporting proteins (RTPs) help the membrane translocation of olfactory receptors. Some odorant receptors, however, did not show enough membrane localization even in the presence of RTP. On the ground of the subcellular localization of the odorant receptors, we hypothesized that these phenomena is due to a rapid internalization of the odorant receptors from the cell membrane. According to inhibition of the internalization, we have succeeded to significantly increase the level of expression of odorant receptors at the cell membrane. These results suggest that the odorant receptor was not tend to retain intracellular region, but susceptible to internalization after disposition to the cell membrane. Moreover, this method may improve the signal/noise ratio of the “artificial olfactory cell”.

Poster session II Poster #382

Effects of sweet taste perception on the hepatic gene expression profile in mice

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Primary tastes are perceived by taste receptors on taste cells and transmitted to gustatory cortices through synapses. On the other hand, it is well known that taste receptors are expressed not only in the taste buds but also in the gastrointestinal tract and that the sweet taste receptors in the enteroendocrine cells could induce hormone secretion. It has been shown that sugars or sweetners are involved in the expression of glucose transporters to secrete incretins, GLP-1 and GIP, via taste receptors in enteroendocrine cells [Margolskee *et al*., *Proc. Natl. Acad. Sci. USA*, 104, 15075-15080 (2007)]. Although the mechanisms of taste perception and transduction of taste signals have been well investigated, the consequent effects on peripheral tissues remain unclear.

Considering that some animals perceive the taste of artificial sweeteners, we undertook experiments to elucidate temporal effects of sweet taste perception in mice on their hepatic gene expression profiles. Using DNA microarray technology, we compared the gene expression profiles in the liver of mice after drinking a saccharin solution and those in mice after drinking water. Mice were given either of the saccharin solution and water for 6h after water and food deprivation for 18h, with the result that serum insulin level was not increased by saccharin solution intake. Hierarchical clustering analysis revealed that gene expression profiles in mice drinking saccharin solution were distinct from those in drinking water. Gene Ontology analysis showed that the expression of lipid metabolism-related genes were significantly changed by drinking the saccharin solution. These results suggest that sweet taste perception itself influences lipid metabolism prior to nutrient absorption in the gut.

Poster session I Poster #205

Soft-diet feeding impaired neurogenesis in the subventricular zone and olfactory-related behavio

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The subventricular zone (SVZ) generates an immense number of neurons even during adulthood. These neurons migrate to the main olfactory bulb (MOB) via the rostral migratory stream (RMS), and upon reaching the MOB they differentiate into granule cells and periglomerular cells. The information broadcast by general odorants is received by the olfactory sensory neurons and transmitted to the MOB. Recent studies have shown that reduction of mastication impairs neurogenesis at the hippocampus and brain functions. Therefore, it is possible that impaired mastication also affects olfactory functions via changes in neurogenesis at the SVZ. However, it is not certain that a reduction of masticatory ability affects neurogenesis at the SVZ and of the olfactory functions. Bromodeoxyuridine-immunoreactive (BrdU-ir) structures in the sagittal section of the SVZ, RMS, MOB and accessory olfactory bulb (AOB) of female adult mice fed a soft diet were studied to explore the effects of reduction of mastication on newly generated neurons at the SVZ and MOB. After 1, 3 and 6 months, the density of BrdU-ir cells in the SVZ and MOB of the soft diet-fed mice was lower than that of the hard diet-fed mice. The olfactory information emitted from general odorants is transmitted to the MOB, while the pheromonal information is transmitted to the AOB. The density of BrdU-ir cells in mice fed a soft diet after 3 and 6 months was lower than that of mice fed a hard diet. The odor preferences of individual female mice to butyric acid were tested in a Plexiglas Y-maze preference apparatus. Avoidance behaviors to butyric acid of the soft diet-fed mice were different from those of the hard diet-fed mice. The present results suggest that feeding with a soft-diet reduces neurogenesis at the SVZ, which in turn reduces olfactory function at the MOB and AOB.

Poster session II Poster #188

Mapping functional circuitry associated with individual olfactory bulb glomeruli using flavoprotein autofluorescence imaging

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The main olfactory system is composed of several distinct subsystems that differ in the chemostimuli to which they respond, the proteins they use to detect and transduce those signals, and the strategies they use to process olfactory information. In contrast to canonical main olfactory bulb (MOB) glomeruli, individual necklace glomeruli of the MOB receive heterogeneous olfactory sensory neuron (OSN) innervation (including from semiochemical-sensitive OSNs expressing the guanylyl cyclase GC-D) and display extensive intrabulbar connections with canonical glomeruli. Thus, they may integrate multiple olfactory signals. To better understand the functional MOB circuitry associated with the necklace glomeruli, we imaged flavoprotein autofluorescence (FA) in the MOB slices upon focal stimulation of identified glomeruli. FA imaging measures changes in endogenous fluorescence produced by mitochondrial flavoproteins upon ATP depletion that accompanies increased metabolic demand. FA signals correspond to neuronal activity and can be followed across synapses, thus facilitating the mapping of functional circuits. Studies in horizontal MOB slices from 3-5 week old mice exhibit a wave of FA signals spreading from the glomerular layer (GL) to the external plexiform layer (EPL) following electrical stimulation (4 sec, 50 Hz, 10-100 μA) of individual necklace or canonical glomeruli. FA signals associated with stimulation of single necklace glomeruli (from mice expressing green fluorescent protein under control of the GC-D gene promoter) were spatially limited compared to canonical glomeruli stimulation. The presence of

10μM gabazine in the bath resulted in a 3 fold-increase in stimulus-dependent FA signal amplitude. A secondary FA wave was also observed from the EPL to the GL in the presence of gabazine. The use of FA imaging should help reveal basic strategies of information processing in the MOB and its subsystems. Support: NIDCD (DC005633), NIGMS (GM008181), NINDS (NS063391).

Contributed talks VI "Interactions" Monday 25 June

Habituation develops at different rates in caterpillars of a specialist and a generalist *Helicoverpa* species and coincides with taste desensitisation

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The two closely related moth species, H. armigera and H. assulta differ significantly in their degree of host-plant specialism. In dual-choice pepper disk arena assays, caterpillars of the two species reared on normal artificial diet were strongly deterred by the alkaloid strychnine. However, caterpillars of the two species reared on artificial diet containing strychnine are insensitive to strychnine. Behavioural experiments on caterpillars subjected to different durations of dietary exposure to strychnine showed that H. armigera did not habituate to strychnine after exposure times of 24 h, 36 h, and 48 h. However, H. assulta displayed habituation to strychnine after exposure during 48 h. After exposure during 72 h, H. armigera did show habituation to strychnine. Electrophysiological tests on caterpillars exposed to strychnine for varying durations revealed that a deterrent-sensitive neuron in the medial sensillum styloconica of the H. armigera and H. assulta caterpillars displayed reduced sensitivity to the deterrent. We conclude that the specialist H. assulta habituated faster to strychnine than the generalist H. armigera and that desensitisation of deterrent-sensitive neurons contributed to the habituation process.

Poster session II Poster #232

Dual agonist and antagonist effects of ligands on the functional response of mOREG

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The identification of antagonists for olfactory receptors (ORs) represents an important challenge that could lead to deep changes in the way to conceive deodorants and perfumes. The recent advance in the field of ORs functional expression has opened the way for the search of specific blockers of receptors that mediate malodor perception. However, there are very few examples of such specific OR antagonism reported so far. One of the best studied, using a calcium imaging-based functional assay, is the eugenol-responding receptor mOREG that was shown to be inhibited by methylisoeugenol (MIEG ; Oka et al. 2007,*Embo J.*,**23**,120-6). However, in a reporter gene-based functional assay, MIEG was also described recently as an agonist of the same OR (Baud et al., 2011, *Biochemistry*, **50**, 843-53).

Here, we used a HEK293-based transient expression system and different functional assays to analyze the agonist and antagonist potential of MIEG on mOREG. This was performed on two variants of the receptor differing by one single amino acid (F252L). We confirmed that MIEG induces a marked reduction of mOREG response to eugenol and vanillin, two potent activators of the receptor. This inhibitory effect was observed on both variants, though more pronounced on the L252 variant in comparison with the F252 variant. A weak activation of the F252 variant was also observed with MIEG tested as an agonist of mOREG when using the most sensitive functional assay. Taken together, these observations led us to propose that a weak, partial agonist such as MIEG may behave as antagonist when challenged against a stronger activator. Therefore we tested the antagonist potential of a series of weak activators of mOREG. We observed that some, but not all, of these compounds induced an inhibition of the vanillin-elicited response on both versions of mOREG. The impact of the F252L mutation on mOREG response to agonists and antagonists will be further discussed.

Poster session I Poster #233

Selective range of activation of OR51E1 with carboxylic acids

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Carboxylic acids represent a major source of body malodors. Among them, isovaleric acid is often pinpointed as a typical foot malodorant molecule. We previously identified and described OR51E1 as a human olfactory receptor activated by isovaleric acid and a series of related carboxylic acids.

In the present study, we have further explored the structure activity relationship of OR51E1 with a new series of carboxylic acids. Using a HEK293-based expression system for this receptor, we were able to demonstrate that the optimal structure for an activator of OR51E1 corresponds to carboxylic acids with a 6 carbon main cycle that harbors a one-carbon bridge between the carbon 1 and 4 of the cycle, such as in bicyclo[2.2.1]- hept-5-ene -2-carboxylic acid or bicylco[2.2.1]-heptane-2-carboxylic acid. Increasing the size of the bridge, such as in bicylco[2.2.2]-octane-2-carboxylic acid results in a decreased potency and intrinsic activity of the molecule. Likewise, replacing the carbon of the bridge by an oxygen such as in 7-oxabicylco[2.2.1]-heptane-2-carboxylic acid reduces the agonist activity on OR51E1. Molecules in which the cycle is methylated were found to be completely inactive on the receptor. Similar results were obtained using 3 different functional assays (either a gene reporter-based assay, a direct measurement of cyclic AMP or a calcium imaging-based assay). Taken together, these observations allow us to propose an olfactophore model for OR51E1.

Symposium 6 "Robotics and artificial chemosensors" Sunday 24 June

From bacterial chemical sensing to rodent maze learning using biomimetic robots: affordance gradients as a universal principle of goal-oriented behavior

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Chemical sensing can be seen as the primitive sense from which all others were derived. I will explore the hypothesis that the functional principle underlying chemically driven actions can be defined as affordance gradients. These continuous gradients either physically exist in the world or are virtual and constructed through behavior and/or the brain. Using an insect based biomimetic multi-modal chemical mapping and localization system I will first analyze the case of bacterial and insect chemotaxis. Using comparative insect and robot experiments I will argue that the specific behavioral strategy of a moth in an odour plume combined with its neuronal representation of the specific stimulation it receives provides for a reconstruction of the complex dynamics of the plume into a mooth gradient. In particular, I will demonstrate the importance of a spatio-temporal coding regime that we hypothesize is realized in the antennal lobe system. Subsequently I will show that internal representations of such continuous gradients can be used to control a wide range of reactive behaviors in the context of autonomous control systems. The drawback of the insect case is that it does assume that rather simple environmental cues define affordance gradients. I will show that the place fields that emerge in the hippocampus of mammals provides for a substrate that can fully virtualize affordance gradients and in this way label the world in terms of the potential for action. I will show that this approach can bootstrap itself towards a fully autonomous planning system for biomimetic robot spatial navigation including chemical localization and mapping in complex environments.

Symposium 6 "Robotics and artificial chemosensors" Sunday 24 June

Biomimetic search strategies

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Macroscopic organisms, such as insects and birds, trying to locate and move towards sources of nutrients, odors, pheromones, etc.. lack local cues because chaotic mixing breaks up regions of high concentration into random and disconnected patches, carried by winds and currents. The sporadic nature of the detections is quantified using Lagrangian transport models. The animal must devise a strategy of movement based upon sporadic cues and partial information. This limits the applicability of gradient-climbing and plume-tracking in the biomimetic design of olfactory robots for applications to the detection of chemical leaks and explosives. A search algorithm, infotaxis, designed to work under sporadic and intermittent conditions will be discussed together with its implementation and some of its recent generalizations.

Symposium 5 "Interspecific chemointeractions" Sunday 24 June

Variation in learning rate in parasitoid wasps: From behavior to brain

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Differences in the expression of learning between closely related species create excellent opportunities to study species-typical learning. We find such an opportunity in our model system of two co-existing *Cotesia* parasitoid species that occupy slightly different niches. In the Netherlands, *Cotesia glomerata* mainly attacks the gregariously feeding caterpillars of the Large Cabbage White *Pieris brassicae*, whereas *Cotesia rubecula* is specialized on the solitarily feeding Small Cabbage White *Pieris rapae*. Both species can learn to associate plant odours with the presence of suitable hosts after an

oviposition experience, but there are some profound interspecific differences in the functional requirements for the formation of long-term memory (LTM). *C. glomerata* learns fast and already forms LTM after a single learning event, whereas *C. rubecula* is a slow learner that needs three spaced learning events to change its plant preference. We argue that this interspecific variation in LTM acquisition reflects a difference in the searching behaviour in nature, related to the distribution of their caterpillar hosts. We have raised selection lines of *C. glomerata* that are different in learning rate. Females from the low learning rate line did not form LTM after a single conditioning trial, but had increased longevity and smaller brains than females from a high learning rate line. Current work focuses on differences in gene expression and in brain morphology. Recently we included a comparison of closely related *Nasonia* parasitoid species. Also in these species we identified species-specific memory consolidation. We prepared a standard brain from series of confocal sections of *N. vitripennis*, and localized octopaminergic and dopaminergic neurons in the brains of these wasps to search for neurons involved in associative learning.

Poster session II Poster #140

Effect of age and Tau pathology on olfactory performances in THY-Tau22 mice.

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Impaired olfaction is a landmark of the early stages of Alzheimer's disease (AD), detected even before cognitive defects. Since 1) cognitive impairments and Tau pathology progression are correlated in both AD patients and experimental models and 2) Tau pathology is enriched in olfactory structures of definite AD cases (Attems et al, 2006), we took advantage of a relevant experimental model of AD (THY-Tau22 ; Schindowski et al. 2006) displaying progressive development of Tau pathology, to study its impact on olfactory performances. Wild-type and transgenic (Tg) mice were studied at three time points : at the age of 4 months when hippocampal Tau pathology is starting and associated with slight cognitive impairments, at mid-course (7 months) and at 12 months when Tau pathology is maximal and cognition is markedly altered. While hyperphosphorylated Tau is detected early in the anterior olfactory nucleus and sharply accumulates from 7 to 12 months in the Tg mice, it only appears in the olfactory bulb at 12 months. Spontaneous olfactory behavior was evaluated in 4- and 7-month mice using a cross habituation task. A significant age and genotype effect was observed. Habituation occurred regardless the age and genotype while differences were observed in spontaneous discrimination between WT and Tg animals. Studies are in progress to characterize further the olfactory discrimination learning abilities in the THY-Tau22 and WT animals in relation to the Tau pathology.

Funded by ANR-10-MALZ-003-01 SOMADOLF and Fondation Recherche Plan Alzheimer.

Symposium 17 "Toward a genetic basis for human olfaction" Tuesday 26 June

Genetic investigation of conscious and subconscious perception of sex steroid-derived odors

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The sex steroid-derived odors, androstenone and androstadienone, are secreted in the sweat and urine of men at higher concentrations than women. These odors are consciously perceived by some people but not detected by others. Exposure to these odors has been shown to alter the level of salivary cortisol in women, leading to the suggestion that these substances modulate social behaviors in humans. We have previously identified an odorant receptor (OR), OR7D4, which is sensitive to these odors. Functional and non-functional variants of OR7D4 co-segregate in humans and we have shown that subjects carrying non-functional variants are less likely to perceive odorous steroids consciously. In ongoing work to match human ORs to candidate odor ligands, we have identified three additional ORs that are sensitive to odorous steroids. As with OR7D4, we find that functional and non-functional variants of these new steroid-sensitive ORs co- segregate in

humans. To test if the conscious perception of odorous steroids and the changes in cortisol induced by them are mediated by the same ORs or through different parallel pathways, we carried out psychophysical studies in female volunteers. We measured cortisol responses to and conscious perception of odorous steroids in a cohort of women in the days surrounding ovulation. We then sequenced the four steroid-sensitive ORs in all subjects. The previously published effect of variation in OR7D4 on conscious perception of androstadienone was replicated in this sample of ovulating women. However, variation in OR7D4 variation did not affect changes in salivary cortisol levels, a measure of a subconscious effect of smelling this steroid. Conversely, variation in another OR did affect changes in salivary cortisol levels, but not the conscious perception of odorous steroids. Conscious and subconscious perception of odorous steroids may be mediated by two molecularly defined subsystems of the human olfactory system.

Poster session II Poster #390

Chorda tympani nerve transection attenuates the rat's avoidance of calcium chloride

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Rats and mice generally prefer to drink water rather than calcium salts solutions in choice tests. The avoidance of calcium salts is presumably due to calcium's unpleasant taste, which humans consider bitter, sour and metallic. However, when extracellular calcium concentrations in the body drop and a need for calcium arises rats demonstrate calcium appetite. These changes in ingestive response to calcium salts depend on orosensory factors, implying the presence of a mechanism for calcium detection in the mouth. To better understand how information about oral calcium is conveyed to the brain, we examined the effects of chorda tympani nerve transection (CTX) on calcium chloride ($CaCl_2$) taste preferences in male Wistar rats using a series of 48-h two-bottle preference test. Whereas control rats avoided $CaCl_2$ at concentrations of 0.1 mM and higher, rats with CTX were indifferent to $CaCl_2$ concentrations up to 10 mM. Rats with CTX had significantly higher preference scores for 0.316 and 3.16 mM $CaCl_2$ than did control rats. Our results suggest that the avoidance of $CaCl_2$ concentrations up to ~10 mM depends solely on the chorda tympani nerve and the input from other gustatory nerves does not come into play until this concentration is reached.

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Poster session II Poster #292

The role of chemical signals in precopulatory reproductive isolation in house mouse superspecies complex *Mus musculus s.lato*

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The present study is aimed to investigate the role of chemical cues in precopulatory reproductive isolation in closely related *Mus* species. Test subjects were two sympatric species which do not hybridize under natural conditions: house mouse (*M.. musculus*) and mound-building mouse (*M.spicilegus*). We used three basic approaches: behavioral, hormonal and immunohistochemical (IHC). To monitor plasma testosterone and corticosterone we used ELISA technique. To visualize activated neurons in vomeronasal organ (VNO) receptor tissue as well as in main olfactory (MOB) and accessory olfactory bulb (AOB) in response to stimulation with odours, Fos IHC was used. To assess behaviour were utilized: two and four preference tests as well as habituation-dishabituation tests. In all tests individuals discriminated con- and heterospecific odours. Both males and females investigated significantly ($p<0.01$) longer opposite sex urine samples of conspecifics versus heterospecifics. Males responded to exposure of estrous female samples of conspecifics with elevated plasma testosterone ($p <0.01$). However we did not observe plasma testosterone response in males when heterospecific female urine was used. In males of different species Fos-immunoreactivity (IR) was recorded in MOB, AOB and in (VNO) epithelium in response to stimulation with urine samples from receptive con- and heterospecific females. In *M. musculus* in response to stimulation with conspecific receptive female urine we

observed Fos-IR in apical and bazal zone of VNO. Using the very same design of experiments in *M.specilegus* we observed Fos-IR in VNO apical zone only. Patterns of activation were different for these two species. There were no activated cells in VNO receptor epithelium in response to stimulation with receptive heterospecific female urine. Data support the hypothesis that chemical cues play a critical role in reproductive isolation of closely related *Mus* species.

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Symposium 4 "Olfactory and taste circuits" Sunday 24 June

Layered reward signaling through octopamine and dopamine in *Drosophila*

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Dopamine is synonymous with reward in mammals. However, despite a recently described involvement in control of motivated behavior in fruit flies, a role for dopamine in reward in insects has been elusive. Instead insect dopamine has been linked to aversive reinforcement and octopamine has been ascribed the role of the reward signal. Using temporal control of neural function in *Drosophila* we find that octopamine mediates the short-term reinforcing effects of sweet taste and also contributes to the state-dependence of memory retrieval. These functions require distinct octopamine receptors and a functional dopaminergic system. In addition, we identify a subset of mushroom body targeted dopaminergic neurons whose activation paired with odor forms robust long-term appetitive olfactory memory. Our observations suggest that dopaminergic neurons, some of which are modulated by octopamine, ultimately represent the reinforcement signals of sweet taste and nutrient value during appetitive learning. Therefore, the positive reinforcement system of flies is more similar to that of mammals than previously envisaged.

Poster session II Poster #234

Effects of levels of food restriction on odor preference learning

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Flavor is a unique stimulus made up of both taste and odor components. Animals prefer flavors that have been paired with a hedonically positive (sweet taste) and/or caloric reinforcer. Previous work showed that conditioned taste preferences are stronger when rats were tested hungry, independent of their deprivation levels during conditioning. Since flavor is made up of both taste and odor components, we looked at the effects of two levels of food restriction on odor preferences in animals. Forty-eight male, Sprague-Dawley rats were restricted to 80% and 90% of their free-feeding body weight. In a flavor preference procedure, both groups were given almond and banana extracts mixed in distilled water and combined with 2% and 20% polycose (a caloric reinforcer) for twenty days. Almond and banana extracts were used in this study because previous work has shown attenuation of conditioned aversions to extracts when rats were made anosmic. This suggests that rats respond to extracts mainly through their odor component. Following training, rats were given both almond and banana in 2% polycose in a two-bottle preference test. The results showed that rats under higher caloric restriction showed a greater preference for the odor cue paired with 20% polycose than rats under lower caloric restriction. This study points out the importance of food deprivation in odor preference learning.

Poster session I Poster #425

Excretion of coffee aroma constituents and their metabolites in human urine

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Coffee is one of the most frequently consumed beverages worldwide. Its effects on human health are frequently and controversially discussed with regard to either positive or adverse effects [1, 2].

More than 800 volatile constituents or flavourants of coffee have been reported [3]. With the exception of caffeine little is known about metabolisation of coffee constituents [4, 5]. Moreover, those few existing studies are often based on animal experiments and not on human metabolisation. Besides, in most of these studies relatively high dosages of single compounds have been applied; therefore it is not sure whether the results are valid for lower concentrations as in foods (coffee) or complex mixtures [4, 5]. To our knowledge no study has been carried out on the metabolisation or excretion of coffee aroma constituents in humans. Accordingly, the aim of the present study was to quantify selected coffee aroma compounds in human urine 1) after three days of coffee abstinence and then 2) after the ingestion of two cups of coffee.

To achieve this goal we applied one- and two-dimensional gas chromatography – mass spectrometry in combination with stable isotope dilution assays. The investigations were carried out both on native urine as well as on urine subjected to glucuronidase assays to also monitor those odorants being conjugated to their corresponding glucuronides.

It was shown that some odorants were present in considerably higher concentrations in human urine after the ingestion of coffee; however, while some compounds were found in their free form, others were heavily metabolized into the corresponding glucuronides.

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Poster session II Poster #426

Odorants in human urine – structural elucidation and quantitative determination

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The volatile and odorous profile of human urine may be a rich source for physiological information (1) and may increase our understanding of metabolization and excretion processes of low-molecular weight compounds originating from e.g. dietary or endogenous sources (2). Odorants of human urine may furthermore play an important role as semiochemicals in human communication as it is common in the animal kingdom (3). Accordingly, the aim of this study was to identify those odorants in native human urine that may represent a characteristic odorant spectrum. Characterization of the odorous substances was carried out by means of combinatory approach involving chemo-analytical and human-sensory tools.

To achieve this goal, one- and two-dimensional high resolution gas chromatography-olfactometry / mass spectrometry (HRGC-O /MS) was applied to identify commonly occurring and potent odorants in human urine, both in freshly obtained native urine, as well as urine that was treated by glucuronidase assays.

Based on retention indices, odour qualities and intensities, and MS-spectra in comparison with references a total of 14 odorants could be detected in the majority of the untreated urine samples, and in the glucuronidase-treated samples 24 odorants. The majority of the identified substances are reported here for the first time. Sensory profiling of native and enzyme-treated urine revealed strong correspondence with the identified substances.

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Contributed talks V "Human olfaction" Monday 25 June

Hedonic responses to food odours in 1-2 year-old toddlers: a longitudinal study

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Olfaction is a salient sensory modality early in life. Neonates show keen olfactory sensitivity and hedonic responsiveness (Schaal, 1988). However, little is known on how olfactory responses develop over the first two years, especially in term of hedonics. Besides, olfaction plays an important role in the perception and appreciation of foods (Yeomans, 2006). Here, we aim to assess hedonic reactivity to food odours in the first two years of life in the context of a longitudinal study on the development of food preferences (OPALINE).

Olfactory tests were conducted on 235 participants at 8, 12, and 22 months. Scented and unscented bottles were presented successively to them. The stimuli consisted in 8 odorants, representing 4 disliked (fish, cabbage, green vegetable, rancid butter) and 4 liked foods (peach/apricot, apple, strawberry, vanilla). Pleasantness of the stimuli was checked by a panel of adults. All tests were videotaped to analyse the following exploration behaviours toward both types of objects: 'no handling' defined as 'no physical contact' and 'mouthing' defined as 'direct contact with perioral and/or perinasal areas'. The duration of each behavioural item was recorded and divided by accessibility duration to provide proportions from which indexes were calculated according to Beauchamp and Moran (1982) and Schwartz et al. (2009).

For the 3 age groups, the results indicated: 1/ longer average duration of 'no handling' of the bottles with unpleasant odours compared to the bottles with pleasant odours; 2/ shorter average duration of 'mouthing' for the bottles with unpleasant odours than for the bottles with pleasant odours; 3/ and some significant correlations across ages for both variables.

These data confirm that toddlers do discriminate the hedonic valence of odours from the age of 8 months. Their hedonic response to this set of odorants appears to be aligned with that of adults, in particular for the unpleasant stimuli.

Poster session II Poster #108

Odor map in the zebrafish olfactory bulb-1: phosphorylated Erk immunohistochemistry

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In many animal species including fishes, olfactory cues in the environment elicit various behaviors essential for survival of individuals and maintenance of species, such as food exploration, predator avoidance, and mate finding. Elucidating odor map in the olfactory bulb (OB) is one of the key steps to understand neural circuit mechanisms underlying these odor-evoked behaviors. Previously, neural activity imaging and electrophysiological studies provided an outline of odor map in the zebrafish olfactory bulb. However, a comprehensive odor map in the OB combined with high-resolution neuroanatomy has yet to be clarified. In this study, we searched for a molecular tool for detection of neural activation in the zebrafish olfactory system and found that the phosphorylation of Erk (MAP kinase) can be used as a reliable marker in both peripheral and central olfactory neurons. Section immunohistochemistry of the olfactory epithelium validated the activation of distinct morphological types of olfactory sensory neurons (OSNs) by different odorants: bile acids (putative social cues) induced Erk phosphorylation in OSNs with long dendrites, whereas amino acids (feeding cues) induced Erk phosphorylation in superficially located OSNs with short dendrites, consistent with previous studies. Whole-mount OB immunohistochemistry from odor-stimulated zebrafish revealed the phosphorylation of Erk in dendrites and somata of OB neurons located in the proximity of particular glomeruli, thereby visualizing the activated glomeruli at high resolution. Further experiments are now in progress to clarify a comprehensive odor map in the zebrafish OB with

various kinds of odor molecules (bile acids, amino acids, nucleotides, prostaglandins, etc.) and biologically relevant cues such as conspecific skin extract which contains a putative alarm pheromone. Our study will provide a fundamental basis for understanding the neural circuit mechanisms linking odor inputs to behavioral outputs.

Poster session I Poster #73

Molecular mechanisms of olfactory detection in *Spodoptera littoralis*: deorphanization of odorant receptors via the *Drosophila* empty neuron system.

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The olfactory sense determines vital steps in insect behaviour, including mate and food search, oviposition site selection and predator/parasitoid avoidance. The Chemical Ecology research group at the Swedish University of Agricultural Sciences in Alnarp and the PISC group at the French Institute for Agricultural research in Versailles, have established the noctuid moth, *Spodoptera littoralis* (the Egyptian Cotton Leafworm) as a model for investigation of noctuid olfaction and chemical ecology. At the molecular level, the fundamental units mediating insect interactions with the olfactory environment are odorant receptor (OR) proteins, which are functionally expressed in odorant receptor neurons within olfactory appendages, primarily the antennae.

One primary research focus has been functional characterization of the *S. littoralis* OR genes, which we recently identified in this species. We are seeking to determine the receptive range of each OR via deorphanisation experiments in a heterologous expression system. Individual ORs are currently being expressed in the Empty Neuron system of the vinegar fly, *Drosophila melanogaster*, to characterize their response profiles by means of single sensillum electrophysiological recordings (SSR). We have also utilized gas chromatographic analysis of plant headspace extracts, coupled to single sensillum recordings (GC-SSR) to analyze the tuning of specific ORs to components of ecologically relevant complex odor blends. Preliminary data demonstrate successful adaptation of these methods to the deorphanisation of *S. littoralis* ORs. These results represent an important step in understanding the molecular mechanisms of olfactory mediated behaviours in *S. littoralis*.

Symposium 13 "Plasticity and modulation in olfactory systems - Linnaeus Symposium" Monday 25 June

Metabolic modulation of olfactory circuit and behavior in *Drosophila*

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The modulation of behavior by internal physiological states is essential for animal survival. Like many other animals, hungry fruit flies exhibit different food searching behaviors. We have identified key olfactory sensory neurons for innate odor preference and discovered that nutrient sensors in these neurons play an important role in appetitive decisions. Our studies indicate that two neuropeptides short neuropeptide F (sNPF) and tachykinin causes presynaptic facilitation and inhibition, respectively, in specific neurons, and the concerted effect controls appetitive behavior in the fruit fly.

Using two-photon calcium imaging, we demonstrate that starvation-triggered behavioral change is accompanied by dramatic shifts in the odor map. The neuropeptide sNPF, a homolog of the mammalian NPY, is highly implicated in hunger signaling and is expressed in *Drosophila* olfactory sensory neurons (OSNs). sNPF signaling in DM1 glomerulus is necessary for the starvation-dependent modulation of both olfactory representation and food search behavior. Furthermore, the sNPF receptor is expressed in OSNs and mediates a feedback enhancement of sensory transmission in DM1. We then investigated starvation modulation in DM5—a glomerulus that mediates innate aversion behavior. Starvation suppresses DM5's sensitivity to odor stimulation, and that this suppression is mediated by tachykinin

signaling. Furthermore, starvation modulation of DM5 also influences food search behavior. Thus, early olfactory processing and appetitive behavior are profoundly controlled by metabolic states in *Drosophila*. Starvation does not simply scale up or down global activity in the antennal lobe. Rather, it upregulates activity in certain sensory channels and downregulate it in others towards a concerted modulation of appetitive behaviors.

Poster session II Poster #274

Electrical stimulation of the human olfactory mucosa

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In animals, electrical stimulation of the olfactory epithelium induces responses at the olfactory bulb. Whether these responses reflect an olfactory percept remains unclear. Electrical stimulation of the human olfactory epithelium has generated mixed results, ranging from diverse olfactory sensations (Uziel *et al*., 1973), to altered perception of actual odorants (Straschill *et al*, 1983), to no sensation at all (Ishimaru *et al*, 1997). We set out to test the hypothesis that modern methods would allow us to generate odor perception by electrical stimulation. We used endoscopic guidance to place a pure-silver stimulating-electrode on the ventral surface of the middle turbinate. An indifferent electrode was placed on the arm. Stimulation was generated using a battery-powered electronic stimulator (current source 0-750 μA), driven by an electro-optically isolated function generator. Mucosal resistance was measured at the beginning of each run, and peak-applied current was ~250 μA. In Experiment I, repeated anodic or cathodic electrical pulses of 40 Hz (square 0.0125 ms pulses, 50% duty cycle) were applied for 2 s, with inter-stimulus-interval of 5 s. These stimuli generated tactile sensations, pain, and visual phosphenes, but failed to generate olfactory sensations. In Experiment II, 2 s odor pulses (L-carvone, decanoic acid, dimethyl sulfide, lactic acid, diacetyl) were delivered into the nostril using an air-dilution olfactometer. Anodic electrical stimulus was applied for 5 s: 3 s before odor onset and during odor delivery. Electrical stimulation failed to influence olfactory perception. In Experiment III, electrical stimulation was applied at different latencies post odor termination (range: 1-10 s). These stimulations also failed to recreate a sensation of smell. In conclusion, electrical stimulation of the middle turbinate did not elicit olfactory sensation or changes in odor perception.

Poster session I Poster #257

A web-based tool for collecting data on olfactory perception

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The world wide web (WWW) provides a unique tool for collecting visual and auditory psychophysics from millions of subjects. This is made easy because the computer, the very same device used to connect to the WWW, can also provide precise visual and auditory stimuli. By contrast, olfactory stimuli cannot be generated by home computer, and this has prevented application of the WWW to collection of olfactory psychophysics. Here we describe a large-scale project consisting of a web page optimized for collecting olfactory psychophysics from participants who either smell odiferous household items, or receive "odor kits" by mail (http://odorspace.weizmann.ac.il/). Participants from around the world can log on, and provide odor estimations. The data can be used to answer questions such as do we all smell the world in the same way? Do odor preferences reflect geography and culture? Do people who smell the world in the same way have other things in common? Moreover, this web-based tool will allow relating odor structure to olfactory perception using an unusually large database. The web page contains various customized tools for graphic presentation of odorant perception and its relation to odorant structure. The website will serve to educate the general public on smell, and will serve as an invaluable tool for researchers. In this poster we will present the design of the website, and provide initial results of data obtained using this tool.

Symposium 12 "No taste, no smell: When the chemical senses are lost " Sunday 24 June

Epidemiology and prognosis of taste and smell loss

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Olfactory disorders are common. Approximately 15% of all adults do exhibit some olfactory disorder while 5% suffer from functional anosmia. With growing age these numbers increase dramatically, even though a large number of affected subjects are primarily not even aware of the disorder. Initially patients even though suffering from an olfactory disorder often complain about changes in food perception and "taste disorders". However, solitary gustatory disorders especially complete ageusia, are rather rare and much less common than olfactory disorders. In most of theses cases even though complaints focus on changes in taste perception an olfactory disorder is the underlying pathology.

Some kind of impairment of gustatory function is present in about 5% of the population. However, studies reporting taste disorders exhibit some variance of data mainly due to the different testing methods applied.

Objective quantification of any of these disorders is mandatory and should be done early in the patient evaluation process

– not only in order to classify an existing disorder but also to get information regarding prognosis. With the use of a validated psychophysical test both, olfactory and gustatory function can be quantified and classified as hyp- or anosmia and hyp- or ageusia respectively. Qualitative disorders cannot yet be measured but can be assessed using questionnaires. The prognosis of taste and smell disorders not only depends on the degree of the disorder but mainly on the etiology of the disorder. Different etiologies, its prognosis and the impact of lateralised olfactory disorders on the prognosis are going to be discussed in detail.

Poster session II Poster #258

Sensory perception and facial reactions of two odors:the case of cis-3-hexen-1-ol and β-ionone

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Fundamentals of human perception can studied by sensory methods; for example, observational studies have shown relations between facial reactions and pleasantness. It has been shown that taste stimuli perceived as equally pleasant differed in facial expressions, meaning that facial reactions can be stimuli specific. Facial reactions have not been reported to be odor specific. The aim of this project was to study relationships between perception of two odors and facial reactions.

Cis-3-hexen-1-ol and β-ionone (aq sol) were prepared in concentrations representing perceptions just above threshold, high above and very high above. Cis-3 was analysed by 10 sensitive panelists and β-ionone by 10 sensitive and 10 non-sensitive. Odor intensity and pleasantness were rated on scales and also verbally described. The test sessions were recorded and then coded according to a selection of FACS units.

Results: Perceived odor intensity differed significantly between stimuli concentrations and was significantly higher for sensitive panelists. Pleasantness was lower for high conc, and no significant difference between sensitive and non-sensitive panelists was obtained. As in earlier studies cis-3 was described as "fresh green", "herbal" and "crushed weeds". Sensitive panelists described β-ionone as other publications: "floral", "perfumey" and "fragrant". Non-sensitives described β-ionone as "musty", "earthy" and "chemical". Sensitive panelists' facial reactions became more intense with higher concentrations, while non-sensitives' did not. No significant differences between the two odor compounds according to facial reactions in sensitive panelists were obtained. It can be concluded that facial reactions induced by the two stimuli mirrored sensitivity and pleasantness, but were not stimuli specific. This might be due to the fact that both odors perceived as pleasant and commonly found in the same products, eg white wine. Future studies on more distant odors are recommended.

Poster session I Poster #259

Olfactory influences on first impressions

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It is reasonably well established that verbal and visual information influences impression formation, but relatively little is known about the way that olfactory information alters a first impression. To explore this, 101 participants were randomly assigned to 1 of 5 groups: Food odor groups (Onion, Lemon), Fragrance groups (Amber, Lily), or a control. They were asked to form an impression of the hypothetical person based on the following stimuli: A gender-neutral silhouette image, a list of personal characteristics, and a vial containing an olfactory stimulus (varying by group). Afterwards, participants were presented with a series of 51 traits that were pre-selected to fall into 4 categories: Cleanliness, pleasantness, masculinity, and femininity. Each trait was rated as to how likely the hypothetical person was to possess it. Participants then assessed all olfactory stimuli on intensity, pleasantness, femininity, masculinity, and cleanliness. The multivariate test of differences in traits describing a person between odor groups using the Wilks Lambda criteria was statistically significant ($F(16, 284.76) = 2.64$; $p = .001$). Follow-up multivariate comparisons showed that the control group was not significantly different from the average of all odors, though food and fragrance odors were different from each other ($F(4,93) = 2.47$; $p = .05$). All of the separate univariate ANOVAs were significant except masculinity, which did not differ between groups. The present data suggest that the absence or presence of an odor is not as important in terms of personality impression as the specific qualities of the odor. Ratings of a person's masculinity were not affected by odor conditions, despite differences observed in ratings of the odors themselves. So, the perception of the hypothetical person did not necessarily differ in the same way that ratings of the odors did, suggesting that the context of a person alters the perception of odors.

Contributed talks IV "Olfactory receptors, ligand interactions and transduction mechanisms" Monday 25 June

Current view on insect odorant receptors

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Insect odorant receptors (ORs) are heterodimers composed of an odorant-specific receptor protein and a co-receptor protein (Orco). The OR proteins possess a 7-transmembrane topology as typical for G protein coupled receptors, but they are inversely orientated within the membrane. Heterologously expressed insect ORs form ionotropic ion channels. In addition to the fast ionotropic response upon odor stimulation they conduct a slowly activating current that relies on metabotropic signaling including cAMP production.

Expression of Orco alone was sufficient to generate a slow current by cAMP stimulation. The sensitivity of Orco to cAMP was seen to be regulated by Orco phosphorylation via protein kinase C. But what is the role of the slow Orco current in insect olfaction? To address this question, we combined single sensillum electrophysiological recordings with microinjection of agents affecting signal transduction pathways and observed the olfactory sensory neuron (OSN) response to odorant stimulation. Increased cAMP production enhanced the OSN response, while reducing the cAMP sensitivity of Orco attenuated the odor response of OSNs. Thus Orco activity contributes to shaping the OSN odor response. Due to the delayed activation kinetics of the Orco current, this shaping reflects preceeding odor signals thereby forming a short-term memory on receptor level.

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Poster session II Poster #260

The aroma of Swedish blueberry (bilberry)

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Bilberry, *Vaccinium myrtillus,* is a wild growing shrub found all over Sweden, mainly in forests. Only a minor part, about 7 %, of the annual production, which is estimated to about 250 million kg, is harvested and utilized for human consumption. However, due to its contents of compounds with beneficial health effects - primarily various antioxidants - and its attractive aroma and flavour characteristics, there has been a considerable increase in the interest of bilberry during the last few years. This presentation focuses on the characterisation of the natural aroma of bilberry fruits by instrumental and sensory methods.

Bilberry fruits were harvested at various locations, from south to north of Sweden, during the year 2011. Aroma compounds in bilberry juice were sampled by dynamic headspace using Tenax traps. Aroma analysis was carried out by GC-FID-O-MS (gas chromatography-flame ionization detection-olfactometry-mass spectrometry). More than 100 odour active volatile compounds were detected by GC-O. Among the most odour active compounds, i.e. compounds with the highest odour intensities, in the olfactograms both fruity, candy-like, grassy, flowery and bilberry notes were perceived. Sensory analysis, by a trained panel, was performed to describe similarities and differences in the aroma of bilberries harvested at various locations.

This study is part of the project “Bärkraft” (the Berry Craft Project), which is partly funded by the European Regional Development Fund.

Poster session I Poster #357

PROP supertaster status mildly correlated with sugar and salt tasting

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We investigated whether the ability to taste 6-*n*-propylthiouracil (PROP) as bitter is related to the ability to taste salt and sweet. Bartoshuk and colleagues (1994) suggested that being a PROP taster involves a higher concentration of fungiform papillae on the tongue and greater sensitivity to all tastes, not just bitterness. We created strips of paper infused with salt water or sugar water and found that taster status had significant predictive ability for the ratings of saltiness and sweetness, with supertasters tasting more than did nontasters and moderate tasters, who did not differ from each other. Bitterness rating of the PROP strip had a significant positive linear relationship with both the sweet and salt strips, but the effect sizes were low and it was common for participants to rate one strip as very strong and one as very weak. This suggests that being a PROP “supertaster” with heightened sensitivity to bitterness is somewhat related to the ability to taste sweet and salt, but other factors besides number of fungiform papillae are important. We suggest that the label of “supertaster” is too simplistic as there can be salt supertasters, sweet supertasters, and bitter supertasters independently. This suggests that taster status is a useful determinant of the ability to taste other flavors, but does not provide a complete picture of a person’s tasting abilities.

Symposium 22 "Odor memory and perception: cells to circuits" Wednesday 27 June

The role of piriform cortical ensembles, pattern recognition and plasticity in odor perception.

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Odor perception is an object oriented process involving recognition of odorant-evoked spatiotemporal patterns in olfactory bulb activity by piriform cortical neural ensembles. Past experience shapes this pattern recognition, which can either enhance or impair cortical and perceptual odor acuity depending on task demands. Based on computational models, experience with co-occurring odorant features helps bind those features through associative synaptic plasticity of association fiber synapse connecting dispersed piriform cortical neurons. This synaptic plasticity helps build cortical representations of experienced odors in the activity of neural ensembles. Work will be presented demonstrating that in rodents, piriform cortical ensemble activity predicts odor perceptual performance across a wide range of discrimination abilities. However, odor perception is not only influenced by past experience, but also by context, expectation and internal state. A potential source of this information is the entorhinal cortex, which receives direct input from the olfactory bulb and piriform cortex, and also sends robust descending fibers back to these areas. Preliminary data will be presented describing top-down control of piriform cortical single-unit and ensemble activity by the lateral entorhinal cortex. Together, the data will describe how plasticity and regional networks help shape piriform cortical activity and the emergence of perceptual odor objects.

Contributed talks VI "Interactions" Monday 25 June

Microbial odors mediate host finding in insect herbivores

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Attraction of the fruit fly *D. melanogaster* to a blend of yeast volatiles with acetic acid as a main attractant compound (Becher et al. 2010, 2011) demonstrates the significance of yeasts in *Drosophila* behavioral ecology. With this in mind, we have investigated the role of yeasts for host finding and oviposition in other insect herbivores. We conclude that plant-yeast-insect interactions are more widespread than previously assumed, and that yeasts and other micro-organisms are important for our understanding of the ecology of insect herbivores and their evolutionary diversification. The traditional bi-trophic plant-insect niche concept must be updated to a tri-trophic niche concept, in order to accomodate for the role of micro-organisms in host-finding. This will be illustrated with results from current studies.

Becher PG, Bengtsson M, Hansson BS, Witzgall P. 2010. Flying the fly: long-range flight behavior of *Drosophila melanogaster* to attractive odors. J chem Ecol 36:599-607
Becher PG, Flick G, Rozpedowska E, Lebreton S, Larsson MC, Hansson BS, Piskur J, Witzgall P, Bengtsson M. 2011. Yeast links the fly to fruit. (submitted)

Poster session II Poster #74

Chemosensory genes from cotton bollworm *Helicoverpa armigera*

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Helicoverpa arimgera (Hubner) is one of the most polyphagous and cosmopolitan pest species, the larvae of which feed on a wide range of plants, including numerous important cultivated crops such as cotton, peanuts, soybeans or maize. The chemosensory system is critical in guiding insect behaviours such as mating, feeding and oviposition. Here we identified chemosensory genes including olfactory receptor (OR), gustatory receptor (GR), ionotropic receptor (IR), odorant

binding protein (OBP) and chemosensory protein (CSP) genes from *H. armigera* genome and transcriptome projects. Then phylogeny analyses were performed to compare the chemosensory genes among *H. armigera* and other insects. The transcription profiles of *H. armigera* chemosensory genes were studied among different tissues, ages and between the sexes. This study will help us build connections between the chemosensory genes and their functions, leading to a better understanding of the molecular mechanism of polyphagous pest chemosensory system and assist in the development of environmentally friendly insect control strategies.

Poster session I Poster #261

Infra smell and ultra smell

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Perceived color relates to a physical axis of wavelength. Humans see light between ~390-750 nm (blue-red), but cannot see under or above these values (UV-IR). Similarly, perceived pitch relates to a physical axis of air vibration. Humans hear between ~20-20K Hz (D#-1-D#9), but cannot hear under or above these values (infrasound – ultrasound). Recently, a physicochemical axis of odorant structure has been linked to odorant pleasantness, low vs. high values indicating unpleasantness vs. pleasantness, respectively. In analogy to vision and audition, we predict that odorants under or above particular values on this spectrum will be odorless. I.e., we predict infra-smell and ultra-smell: odorants that smell so bad or so good, that they don't smell at all. To test this, we first obtained pleasantness estimates for 64 odorants from 14 subjects, and optimized our prediction of pleasantness from structure to obtain a correlation of $r=.83$, $p<0.0001$. I.e., we could predict pleasantness from structure. We next used this model to predict the pleasantness of 1492 molecules. Of these, 26 were listed by the GoodScents database as "odorless". In stark contrast to our prediction, these molecules did not inhabit the extremes of our axis, but rather were concentrated in the middle. This result may reflect one of three possibilities: First, odors listed in this database as "odorless" may in fact have an odor. An initial examination of 13 molecules suggests that this indeed may be the case. A second explanation may be that our database of 1492 molecules is skewed in olfactory space. A third alternative explanation for this initial result may be that our theory is wrong. To provide a definitive test of this theory, we will apply the model to a much larger selection of molecules, and verify odorlessness psychophysically, rather than rely on published descriptors. Together, this approach may guide a theoretically driven investigation of the natural statistics of the olfactory world.

Poster session I Poster #113

Olfactory neural circuitry mediating prostaglandin F2α-evoked sexual behavior in male zebrafish

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Pheromones are conspecific olfactory cues which elicit various social behaviors in many animal species including fishes. For example, prostaglandin F2α (PGF2α) has been proposed as a sex pheromone in teleosts. In goldfish, PGF2α is released from females and evokes typical reproductive responses in males (e.g., increase of swimming activity and nudging). However, it remains largely unknown which types of olfactory sensory neurons (OSNs) are activated by PGF2α and how its pheromonal information is transferred from the olfactory epithelium to the olfactory bulb and further into higher brain centers to evoke the sexual behavior. In this study, we used zebrafish to address these issues because of its genetic amenability for visualization and manipulation of selective neural circuits. To identify PGF2α-activated OSNs and central neurons along the olfactory pathway, we immunohistochemically examined the phosphorylation of Erk (MAP kinase), a reliable marker for detecting neuronal activation in zebrafish. Upon PGF2α stimulation, pErk immunoreactivity was detected in a small subset of ciliated OSNs in the olfactory epithelium. In the olfactory bulb, pErk-positive signal was specifically observed in a ventromedial glomerulus. In addition, PGF2α-activated pErk-positive neurons were found in several regions in the ventral forebrain. These results provide a neuroanatomical basis for the

olfactory neural circuitry mediating PGF2α-induced sexual behavior in zebrafish. Further analysis of functional significance of the neural circuitry identified by anti-pErk immunohistochemistry is now in progress with an integrated approach combining behavioral and genetic methods.

Symposium 8 "Central mechanisms of taste learning and memory" Sunday 24 June

Neural substrate of conditioned taste aversion -Roles of amygdala and reward system in acquisition and expression-

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One of the characteristics of conditioned taste aversion (CTA) is a hedonic shift for the conditioned stimulus (CS) from positive to negative after the CS is associated with malaise-inducing unconditioned stimulus (US). The flow of CS information from the amygdala to the reward system plays a crucial role in the acquisition and expression of CTA. The CS induced robust activity in the basolateral nucleus of the amygdala (BLA) where only a modest activity was induced by the CS before association with the US. The CS activates the neural projections from the BLA to the CeA and extended amygdala. The BLA, CeA and extended amygdala neurons send axons to the nucleus accumbens (NAc) which has GABAergic connection to the ventral pallidum (VP). Re-exposure to the CS after CTA acquisition enhanced activities of the efferents from the NAc to the VP, and increased the extracellular GABA release in the VP. The blockade of $GABA_A$ receptors by bicuculline in the VP disrupted the expression of CTA with the elimination of aversive responses to the CS, suggesting that the GABAergic neurotransmission in the VP is essential in expression of aversive responses to the CS. This notion was supported by the finding that microinjections of muscimol, a $GABA_A$ receptor agonist, induced robust aversive taste reactivity and modest ingestive reactivity after the voluntary intake or the intraoral infusion of normally preferred water or saccharin solution. Increased GABAergic transmission in the VP activates various brain regions responsible for the aversive taste reactivity, including the parvicellular subdivision of the intermediate nucleus of the NTS (iNTSpc), a region strongly activated in association with CTA expression. The iNTSpc, which is proved to receive direct projection from the amygdala, might receive inputs from the VP to exert aversive reactions by exposure to a conditioned aversive taste. The neural networks including the BLA, NAc and VP are crucial in the hedonic evaluation of ingested food.

Poster session I Poster #75

EXPRESSO: real-time automated recording and analysis of feeding behavior in *Drosophila melanogaster*

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The regulation of food intake is controlled by the nervous system, which evaluates external chemosensory information and internal physiological state. In the fly, *Drosophila melanogaster*, feeding state regulates attractiveness to food odours and responsiveness to sugars. After fasting, flies increase their food intake to compensate for the energy deficiency incurred. Although feeding and chemoattractive behaviors have been studied intensively in flies, the neural circuits that connect hunger, satiety, and food quality evaluation are poorly understood.

In the last few years, development of multiple genetic tools that can control neural activity has made *Drosophila melanogaster* an important model organism for the study of neural circuits and function. Although these genetic manipulations are very powerful, behavioral assays that measure their effects are limited because of the absence of thorough, quantitative, and automated methods. Currently there is no automated system to analyze fly feeding behavior in real time.

We have developed a new method based on the previously described capillary feeder (café) assay to measure liquid food consumed by individual flies in real time. Our method works by measuring light intensity changes in a glass capillary filled with liquid food. When a fly consumes the liquid food, the decrease in liquid level alters light intensity, which is captured by an array of photodiodes. A computer reads the signal generated by the photodiodes and custom software converts this signal to the amount of liquid food consumed in real time. From the real time feeding data we can calculate meal size, meal duration and meal speed of an individual fly tested. We believe our automated system will enhance the temporal analysis of fly feeding behavior and facilitate genetic screens for flies that are defective in regulating food satiety and attraction.

Poster session I Poster #255

The tip of the nose in the brain

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We are all familiar with the phenomena where we smell an odor, are sure that we know what it is, but are unable to name it. Here we set out to use fMRI to uncover the brain organization of this "tip of the nose" sensation. In a 3-Tesla Siemens MRI scanner (TR=1.5 sec, TE=23ms) 16 subjects were presented with 1 of 30 familiar odors for 6 seconds, and after 12 seconds they had to say whether they felt odor identity was (i) known, (ii) on the tip of their tongue (TOT), (iii) familiar or (iv) not known. First, we preformed region of interest (ROI) analysis. ROIs included olfactory (bilateral piriform cortex and orbitofrontal cortex), memory (bilateral hippocampus) and verbal (Inferior Frontal Gyrus and Superior Temporal Gyrus) related areas. This analysis revealed that only left piriform cortex discriminated between degrees of feeling of knowing ($F(3,15) = 4.83$, $p<0.0053$). Post-hoc comparisons revealed increased left piriform cortex activity for "Know" ($p<0.012$), "TOT" ($p<0.0043$) and "Familiar" ($p<0.026$) than for "Don't know". Next, we tested whether coactivation upon these ROIs changed according to different feeling of knowing. Analysis revealed that coactivation between left piriform and left hippocampus was higher for "Know" than for "TOT" ($p<0.017$), "Familiar" ($p<0.0046$) and "Don't Know" ($p<0.0037$). These results indicate that feeling of knowing odor identity is reflected in olfactory and memory regions. More so, as opposed to feeling of knowing of visual objects, verbal-related regions are not involved in feeling of knowing for odors.

Poster session I Poster #235

Morphological and histochemical studies of the nasal cavity and fused olfactory bulb of the Zebra finch, *Taeniopygia guttata*

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Because the zebra finch (*Taeniopygia guttata*) is commercially available, easy to breed in captivity and robust to anaesthesia, this bird has become the measure model for many neuroscience studies especially for human speech and learning. However, until now, only a few studies have investigated the chemical sensory apparatus of this bird, so little is known of the types of chemical senses that it uses. Thus, we analyzed the anatomical and histological properties of the nasal cavity and olfactory bulb (OB) of the zebra finch to investigate its functional level of the olfaction. In the nasal cavity of the zebra finch, although the anterior and maxillary conchae were clearly observed, there was obscure structure equivalent to the posterior concha. The olfactory epithelium of the zebra finch occupied remarkably small area of the posterior concha and had the histological and ultrastructural features of the olfactory receptor cells like already reported other avian species. The ratio of the OB size to that of the cerebral hemisphere was very small. The left and right OB were completely fused and located on the ventral side of the anterior extremity of the cerebrum. Several types of the lectin, which bind the olfactory nerve of vertebrates, were positively bound to the olfactory nerve layer and glomerular layer of the OB. These morphological and histochemical properties were reflected like the bird of the passeriformes, such as the Japanese and/or American crows and the brown-eared bulbul. Our results suggested that the zebra finch has a "limited" sense of olfaction, as same as other passeriforme avians. Moreover, zebra finch's "fused" OB may offer a unique model for studying the evolution and development of the vertebrate olfactory system.

Poster session II Poster #236

Neuronal mechanisms underlying olfactory preference change depending upon odor concentration

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The same odorant can induce attractive and repulsive responses depending upon its concentration in various animals. An odorant indole, for example, is perceived as floral at low concentrations, but has a putrid odor when concentrated. However, little is known about the neuronal basis of the preference change depending upon odor concentration. Here we demonstrate the neuronal mechanisms of the phenomenon in the nematode *C. elegans*, whose nervous system structure is the most thoroughly described of any animals.

C. elegans avoids high concentrations of odorants such as isoamyl alcohol that are attractive at low concentrations. We previously reported that *C. elegans* approaches the odorant using two behavioral strategies, klinokinesis and klinotaxis. Behavioral analyses and computer simulation revealed that the behavioral change depending upon odor concentration is primarily generated by klinokinesis, in which worms change the frequency of turning by sensing the odor gradient.

Laser ablation and genetic analyses revealed that distinct groups of sensory neurons are required for the attraction to and avoidance of an odorant at different concentrations: AWC sensory neurons are known to mediate attraction to lower concentrations of isoamyl alcohol, whereas AWB, ADL, and, in particular, ASH sensory neurons are required for avoidance of higher concentrations of it. Moreover, calcium imaging revealed that activity patterns of these sensory neurons change dramatically depending upon odor concentration: AWC neuron responds to only lower concentrations of isoamyl alcohol, whereas ASH neurons respond to only higher concentrations of it. Hence, our study suggests that odor concentration coding in *C. elegans* mostly conforms to the labeled-line principle, in which the quality of a stimulus is encoded by cells that respond preferentially to that stimulus.

Contributed talks II "Gustation" Monday 25 June

Coding of taste signals in the mouse periphery

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Taste information such as sweet, salty, umami, sour and bitter is important for evaluating chemical components in foods and drinks. Each of these taste qualities may be devoted to detection of nutritious and poisonous contents; sweet for carbohydrate sources of calories, salty for minerals, umami for protein and amino acids contents, sour for ripeness of fruits and spoiled foods, bitter for harmful compounds. Encoding of these taste qualities begins with taste receptors on the apical membrane of taste receptor cells. Activation of taste receptor leads to depolarization of the taste receptor cell, transmitter release, and activation of gustatory afferent nerve fibers. During this process, the sensitivity of taste receptor cells and the connection between taste receptor cells and gustatory nerve fibers may be critical for coding of taste signals. Recently, we recorded taste responses from mouse fungiform taste bud cells generating action potentials and compared them with those of chorda tympani nerve fibers. Both taste bud cells and fibers fell into several groups with different responsiveness to basic taste stimuli and the occurrence of each group did not significantly differ between taste bud cells and fibers. In addition, each of sweet-, salt-, and umami- responsive types of cells and fibers has similar subtypes segregated by their response profiles and susceptibilities to receptor inhibitors and antagonists. These data suggest selective connection between corresponding classes of taste bud cells expressing particular taste receptors and gustatory nerve fibers, thus forming coding channels for taste perception. Each of coding channels may provide information on specific taste and more slight differences between taste compounds.

Symposium 4 "Olfactory and taste circuits" Sunday 24 June

Olfactory circuits in zebrafish

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Zebrafish has become one of the most useful model organisms in neurobiology. In addition to its general advantageous properties (external fertilization, rapid development, transparency of embryos, etc.), zebrafish is amenable to various genetic engineering technologies such as transgenesis, mutagenesis, gene knockdown/knockout, and transposon-mediated gene transfer. Our transgenic approach unraveled two segregated neural pathways originating from ciliated and microvillous sensory neurons in the olfactory epithelium to distinct regions of the olfactory bulb, which likely convey different types of olfactory information (e.g. pheromones and odorants). Furthermore, the two basic principles (one neuron - one receptor rule and axon convergence to target glomeruli) are essentially preserved also in zebrafish, rendering this organism a suitable model vertebrate for the olfactory research. In this talk, I will summarize recent advances in our knowledge on functional architecture of the zebrafish olfactory circuits mediating specific odor-induced behaviors. In particular, I will focus on molecular genetic dissection of the neural elements involved in the attraction to food odorants, the aversion from alarm pheromones, and the social response to sex pheromones.

Poster session II Poster #276

An approach to explain the change of odor preference by blending using the calculated molecular features of the odor components

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Pleasant odors of flowers and foods, etc. are often mixtures of many odorous molecules. This work aims to search the change of odor preference by blending of odorants and it is an approach to explain the change using the calculated molecular features of their components.

The lavender essential oil and the sage essential oil were used for this work. The preferences of each essential oil and the blended oil of the two were evaluated. Two odor presentation methods were used, one was the static bottle method and the other was dynamic blending-machine method. The results were not corresponding in the two olfactory evaluation methods. However, when the static method was used, it was found that 11 human subjects in the 13 preferred the blended oil than the lavender essential oil and sage essential oil. Though the essential oils themselves are the mixture of many odorants, the preference of odor may increase by blending the two essential oils. Further olfactory evaluation is under way.

On the other hand, an attempt was made to describe the two essential oils and the blended oil using the calculated chemical features of their component molecules. Five main component molecules of the lavender essential oil, including linalool and linalyl acetate, were selected. Eight main components of the sage essential oil, including alpha-tujone, were selected. The flexibilities, electric charges, IR and Raman spectra of the selected component molecules were calculated using Gaussian 03W®. The IR spectra of the component molecules were cut by $1cm^{-1}$ width in the range of 0-4000 cm^{-1} , and they were summed up in consideration of the ratio of the amounts contained in the both essential oils. The spectroscopic property is selected, because it reflects functional groups of molecular structures and is convenient parameter for adding up.

It is planned to complete the odor models that describe the essential oils and the blended oil, and that explain the preference of the blended oil.

Poster session I Poster #277

Smelling Phenomenal: rethinking the distinction between access and phenomenal consciousness

Benjamin D Young

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Olfaction suggests a new treatment of phenomenal consciousness and awareness that questions the viability of Block's distinction between these states of consciousness. Block (1995) argues that the concept of consciousness is not a cluster concept containing relevantly similar concepts but a mongrel containing different kinds of states. The two states that he distinguishes are access-consciousness (awareness) and phenomenal-consciousness (qualitative character) (1993,1995,2001,2007, 2008,2009,2011). However, his distinction between these kinds of consciousness is conceptually ambiguous (Rosenthal,2002,2007,2009,2010) and challenged as incapable of scientific investigation (Kouider et al.,

2012). Thus, I offer a novel theory of phenomenal consciousness and awareness using empirical evidence that shows olfactory phenomenal consciousness occurs without conscious awareness, but phenomenal consciousness is necessarily constitutive of conscious awareness. Evidence that olfactory sensory states have a qualitatively character in the absence of awareness derives from research on blind smell (Schwartz,1994,2000; Sobel,1999), mate selection (Beauchamp et al.,

1985; Jacob et al.,2002; Ober, et al.,1997; Wedekind et al., 1995; Yamazaki et al.,1979; Yamaguchi et al.,1981; Ehman, et al.,2001), the selection of social preference for social interaction and acquaintances (Herz & Schooler,2002; Jacob et al.,2002; Li et al.,2007), as well as the role of olfactory deficits in causing affective disorders (Deems, et al.,1991; Miwa et al.,2001). Furthermore, evidence that olfactory awareness is always phenomenally conscious derives from the sniffing (Bensafi et al.,2003) and cortical activation patterns (Bensafi et al.,2007; Rinck et al.,2009) during olfactory imagery experiments (Algom & Cain,1991; Stevenson et al.,2005). Olfactory consciousness provides a new treatment of Block's distinction that does not suffer from his definitional ambiguity and scientifically unverifiable nature.

Poster session II Poster #76

Herbivore-induced de novo synthesized volatile compounds repel oviposition in the moth *Spodoptera littoralis*

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Nocturnal herbivores use volatile cues during selection of suitable host plants for feeding, mating and oviposition. Female Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) moths have been shown to avoid oviposition on damaged cotton plants as well as on undamaged plants having herbivore-damaged cotton plant neighbours. Herbivore-induced plant volatiles (HIPVs) have thus been shown to affect oviposition behaviour in *S. littoralis,* but the volatile compounds responsible for oviposition avoidance are unknown. Our behavioural experiments show that exposure to HIPVs emitted directly from damaged plants or to headspace collections thereof reduced the preference of *S. littoralis* to oviposit on undamaged plant under both laboratory and field conditions. Gas chromatography-electroantennographic detection (GC-EAD) studies using headspace collections reveal that antennae of mated female *S. littoralis* moths detected

18-compounds belonging to several chemical classes. Further laboratory and field experiments show that a mixture of seven *de novo* produced compounds (E-*β*-ocimene, (E, E)-2,4,6-trimethyl-1,3,7,11-tridecatetraene (TMTT), (E)-2,4-dimethyl-1,3,7-nonatriene (DMNT), linalool, (E, E)-α-farnesene, (Z)-3-hexenyl acetate and indole) repel *S. littoralis* from oviposition on undamaged plants. No effect on oviposition could be established for the remaining eleven compounds.

Poster session I Poster #77

Characterization of pheromone receptor genes in the turnip moth, *Agrotis segetum*

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Mate recognition in moths involves the production of sex pheromones by females and their detection by specific receptors on male antennae. The female turnip moth, *Agrotis segetum* (Noctuidae), produces a mixture of chemically related pheromone components including (*Z*)-5-decenyl, (Z)-5-dodecenyl, (*Z*)-7-dodecenyl and (*Z*)-9-tetradecenyl acetate (Z5-10:OAc, Z5-12:OAc, Z7-12:OAc and Z9-14:OAc). The pheromone components and the behavioral antagonist (*Z*)-5-decenol (Z5-10:OH) elicit specific responses from receptor cells on the male antenna. This set of similar ligands and corresponding specific receptors makes the turnip moth system a suitable model to unravel the molecular basis of specific ligand-receptor interaction. We cloned eight candidate pheromone receptor (PR) genes and the *Orco* gene from *A. segetum* by degenerate primer based RT-PCR and RACE PCR. These genes were named *AsegOR1-8* and *Aseg\Orco*. By construction of a phylogenetic tree including these genes and functionally characterized PRs from other moth species, we found that the candidate genes clustered in different expansions of previously described PR genes from the noctuid *Heliothis virescens*. Notably, five of them cluster in the expansion of HR16 and show high amino acid sequence identity (71.0-97.7%). This suggests a rapid evolution of PR genes in *A. segetum*. Using the *Xenopus laevis* oocyte expression system and two-electrode voltage clamp recording, we identified the ligands of two PR candidates from the HR16 expansion. AsegOR1 is specifically responsive to *Z*5-10:OAc, the pheromone component that evokes the largest electrophysiological response *in vivo*; whereas AsegOR6, which is 82.9% identical to AsegOR1, responds specifically to the corresponding alcohol, the behavioral antagonist *Z*5-10:OH. Substitution of a few amino acids accounts for changes in ligand specificity. Characterization of other PR candidates is underway as part of an extensive study of the molecular basis of receptor-ligand interactions.

Poster session II Poster #78

Rescue® stink bug trap for both outdoor and indoor use: Olfaction and vision in action

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The brown marmorated stink bug (BMSB), *Halyomorpha halys*, was introduced from Asia into the mid-Atlantic region, USA, in the mid 1990s. It has been recorded in a total of 35 states. In recent years, the BMSB emerged as a severe pest of fruit and many other crops across the mid-Atlantic region. In addition, this invasive species is a serious nuisance for homeowners and businesses, as it overwinters in residential houses. Recent USDA studies indicated that BMSBs are strongly attracted to a known stink bug pheromone, methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate. This pheromone will have a great potential for monitoring and mass-trapping of this serious invasive stink bug, if a simple and efficient stink bug trap is commercially available.

In order to fulfill such an urgent need, Sterling International, Inc. developed a pheromone-based outdoor Rescue® stink bug trap for both consumer and agricultural markets in 2011. This outdoor trap catches not only both sexes of stink bug adults, but also the nymphs. It can also be used during the overwintering season as a trapping device for indoor use when attached to our newly developed the “Stink Bug LED Light” for the home-owners. Both the outdoor stink bug trap and indoor LED Light are now available at all the major USA consumer retailers such as Home Deport, Wal-Mart, Costco, ACE hardware, True Values etc. The “Stink Bug LED Light” trapping system is also efficient for catching other common indoor nuisance insects such as the multicolored Asian lady beetle (*Harmonia axyridis*), the boxelder bug (*Boisea trivittatus*) and the kudzu bug *(Megacopta cribraria*) during the overwintering season.

Symposium 23 "Evolution of chemosensory systems " Wednesday 27 June

Neural circuits of *C. elegans* olfactory learning

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C. elegans, with the welldefined connectivity of the 302 neurons in its entire nervous system, offers an opportunity to dissect the function of neural circuits for learning. The "wiring diagram" has facilitated the functional mapping of hardwired circuits underlying behaviors. We and others have also identified a second type of circuits, "the invisible wiring diagram", which regulates behaviors, including learning, on the basis of peptides, and acts in a way that is largely independent of synaptic connections. Intriguingly, our recent work has uncovered a third type of circuits, "the topographic circuits", which is based on the subcellular anatomical organization of the synapses within neuronal processes. These findings together underscore the computational potential of the small C. elegans nervous system in regulating behaviors and experiencedependent plasticity.

Poster session II Poster #358

The effects of sucrose and saccharin solution intake on body weight regulation and the sweet taste receptor expression in both male and female rats

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Soft drinks, originally sweetened mainly by sugars, are now also sweetened by artificial sweeteners. Consumption of soft drinks has increased the last three decades and is partly responsible for the epidemic-like increase in obesity, but the findings are mixed. In this study, we investigated whether changes in body weight and sweet taste receptor expression under the long-term intake of sucrose and saccharin solutions and whether sex-associated differences exist. Both male and female rats received either sucrose or saccharin solution and standard rat chow for ten weeks. Rats receiving water alone and standard chow were controls. Food intake and body weight were followed and mRNA levels of sweet taste receptor T1R2 and T1R3 (in the taste buds and the hypothalamus) were measured. All rats offered the sucrose solutions increased their total caloric intake and body weight. The increased caloric intake occurred despite the fact that the rats consumed less chow. Male rats offered with saccharin solution increased their food intake and body weight, but the female rats showed no significant difference with controls. In addition, consumption of sucrose solution resulted in up-regulated sweet taste receptor T1R2 and T1R3 expression in the taste buds in both male and female rats. Consumption of saccharin solution only induced TIR3 mRNA up-regulated in male taste buds. However, in the hypothalamus, there is no significant difference among all groups. These results show that chronic intake of sweet taste solution (sucrose or saccharin) altered the expression of sweet taste receptor in the taste buds and can help to explain the higher tendency of males to suffer from obesity-linked disorders under saccharin solution intake and the possible mechanisms of the excessive consumption of sucrose solution contribute to the incidence of obesity in both male and female rats. The present study is supported by the National Natural Science Foundation of China (No.31171052 and 31000518).

Poster session I Poster #419

Olfactory modulation of visual temporal processing

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Perception is thought to consist of fast 'snapshots' of the world on a need-to know basis. We ask if additional information from the olfactory channel, which naturally conveys object identities, enhances the temporal encodings in the visual system as indexed by subjective duration and critical flicker fusion (CFF) frequency. Using two alternative forced choice method, we find that a smell lengthens the subjective duration of a sensory congruent visual object and enhances its visibility at frequencies near CFF in a manner independent of top-down cognitive control. These results indicate that olfaction modulates visual temporal processing at the object representation level, and hence shed light on the neural timing of multisensory events.

Poster session I Poster #99

Novel structural features of insect odorant binding proteins and their involvement in ligand binding

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Odorant binding proteins (OBPs), present in the sensillum lymph of insect antennae, capture signal molecules (pheromones, host volatiles) and ferry them to the olfactory receptors on the neurons (ORs). However, the molecular basis of recognition and selectivity between the signals and OBPs is still not fully understood. We have taken a whole genome approach to characterise genes encoding OBPs and examined their expression profiles, binding properties and crystal structures. We have expressed and purified OBPs from well-studied systems such as bombykol/BmorPBP1/BmorGOBP2 of the silk moth *Bombyx mori*, 11-cis-vaccenyl acetate/LUSH/PBPs of the fruit fly *Drosophila melanogaster*, indole/AgamOBPs of the mosquito *Anopheles gambiae*, 1-Octen-3-ol/GmmOBP14 of the tsetse fly *Glossina morsitans morsitans* and (*E*)-beta-farnesene/ApisOBPs of the pea aphid *Acyrthosiphon pisum*. The protein core of all of the OBPs has a similar folding structure and is mostly helical with differences in the length and structure of the C-terminus, the number of disulphide bonds, the residues involved in ligand binding and the flexibility of the binding cavity. OBPs with the characteristic 6 cysteines can be divided into short, medium and long C-terminus subclasses and the C-terminus of proteins such as BmorGOBP2 and AgamOBP7 form an extra 7th α-helix. AgamOBP7 also has an extra 4th disulphide bond which holds its C-terminus to form a wall of the binding cavity. X-ray structures of BmorGOBP2 show that the bound ligand, bombykol, adopts a different conformation from that found when it binds to BmorPBP1, with binding to BmorGOBP2 involving a hydrogen bond to Arg110 rather than to Ser56, as found for BmorPBP1. There are no general rules to describe the ligand specificity of OBPs. Each OBP displays its binding capacity in its own conformation and may interact with other OBPs. It is presumably a combination of these interactions and interaction with ORs that facilitate the observed behavioural specificity of insects to the signal molecules.

· Zhou JJ et al. (2009) Characterisation of Bombyx mori Odorant-binding proteins reveals that a general odorant-binding protein discriminates between sex pheromone components. *J Mol Biol*. 389:529-545.
· Zhou JJ. (2010) Odorant-binding proteins in insects. *Vitam Horm*. 83:241-272.
· Lagarde A et al., (2011) The crystal structure of odorant binding protein 7 from Anopheles gambiae exhibits an outstanding adaptability of its binding site. *J Mol Biol*. 414:401-412.

Poster session II Poster #112

Opto- and pharmacogenetic analysis of juxtaglomerular interneuron function in the zebrafish olfactory bulb

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Juxtaglomerular neurons are a large and heterogeneous population of interneurons in the olfactory bulb (OB) that mediate feed-forward and feed-back inhibition of interneurons and of output neurons, the mitral cells (MCs). It is, however, poorly understood how they shape odor-encoding activity patterns across MCs and contribute to neuronal computations in the OB. We manipulated the activity of juxtaglomerular neurons using a pharmacogenetic approach and analyzed the effect on MC activity patterns by whole-cell recordings and temporally deconvolved 2-photon calcium imaging in the adult zebrafish brain. The allatostatin receptor (AlstR), which activates hyperpolarizing GIRK channels when bound by its ligand, allatostatin, was expressed in subsets of juxtaglomerular neurons. Bath-application of allatostatin at low concentrations reversibly decreased input resistance and firing rates of AlstR-expressing neurons and, in many cases, silenced them completely. Subsets of AlstR- negative MCs and interneurons showed significantly increased spontaneous firing, while other subsets showed decreased activity. Odor-evoked population activity patterns dramatically changed in an odor- and neuron-dependent manner. On average, odor responses were increased in the presence of allatostatin. No effects of allatostatin were observed in control fish. To further analyze the connectivity and synaptic effects of juxtaglomerular neurons, we expressed channelrhodopsin-2 (Chr2) instead of AlstR and optically stimulated juxtaglomerular neurons using a digital micromirror device (DMD) or 2-photon excitation of Chr2 in individual neurons. Results indicate strong inhibitory effects of juxtaglomerular neurons on MCs and other interneurons. Preliminary data suggest sparse and strong connectivity from juxtaglomerular neurons onto their targets. Together, these results show that juxtaglomerular interneurons strongly shape OB output activity patterns by direct inhibition, as well as by dis-inhibition.

Poster session II Poster #420

Crucial role of copper in detection of metal-coordinating odorants

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Odorant receptors (ORs) in olfactory sensory neurons (OSNs) mediate detection of volatile odorants. Divalent sulfur compounds such as thiols and thioethers are extremely potent odorants. We identify a mouse OR, MOR244-3, robustly responding to (methylthio)methanethiol ($MeSCH_2SH$; MTMT) in heterologous cells. Found specifically in male mouse urine, strong-smelling MTMT [human threshold 100 parts per billion (p.p.b.)] is a semiochemical that attracts female mice. Non-adjacent thiol and thioether groups in MTMT suggest involvement of a chelated metal-complex in MOR244-3 activation. Metal ion involvement in thiol-OR interactionswas previously proposed, but whether these ions change thiol-mediated OR activation remained unknown. We show that copper ion among all metal ions tested is required for robust activation of MOR244-3 toward p.p.b. levels of MTMT, structurally-related sulfur compounds and other metal-coordinating odorants, e.g., strong-smelling *trans*-cyclooctene, among >125 compounds tested. Copper chelator (tetraethylenepentamine, TEPA) addition abolishes the response of MOR244-3 to MTMT. Histidine 105, located in the third transmembrane domain near the extracellular side, is proposed to serve as a copper-coordinating residue mediating interaction with the MTMT-copper complex. Electrophysiological recordings of the OSNs in the septal organ (SO), abundantly expressing MOR244-3, revealed neurons responding to MTMT. Addition of copper ion and chelator TEPA, respectively enhanced and reduced the response of some MTMT-responding neurons, demonstrating the physiological relevance of copper ion in olfaction. In a behavioral context, an olfactory discrimination assay showed that mice injected with TEPA failed to discriminate MTMT. This study establishes the role of metal ions in mammalian odor detection by ORs. www.pnas.org/cgi/doi/10.1073/pnas.1111297109

Poster session I Poster #79

Eucalyptol, the general odorant sensed by sex-specific sensilla in the cockroach, *Periplaneta americana.*

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Eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane) is known as the major monoterpene of eucalyptus oil, abundant plant volatile in natural cockroach habitats. Repellent properties of the substance were shown for a number of insect species including juveniles and females of American cockroach (Scriven and Meloan,1984). However, adult cockroach males respond to this odor in a different way. Unlike the most plant-derived odors it is sensed by neurons housed in male sex-specific sensilla tuned to pheromone components. The pheromone sensitive sensilla of swB type responded to eucalyptol stimulation with an average firing rate of 29.7±4.1 spikes per recording. Octopamine treatment, which was shown to increase firing response of these sensilla to both main pheromone components, periplanones A and B, did not affect eucalyptol responses. At the same time, amplitude of electroantennogram (EAG) in response to eucalyptol decreased similarly with EAG responses to periplanones. Behavioral responses of untreated adult males to eucalyptol in two-choice bioassay were neutral. However, males, aroused by nearby placed calling females, were attracted to eucalyptol. Similar effect was found under octopamine ingestion. Simultaneous presentation of periplanone B and eucalyptol resulted in slightly increased threshold for wing-raising behavioral response to pheromone. It is known (Seelinger, 1984) that Periplaneta males occupy raised "perching" places to monitor for female sex pheromone. Eucalyptol is suggested to serve as orientation cue for the male cockroach, leading it to a "perching" site.

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Symposium 20 "Aquatic olfaction" Tuesday 26 June

The neural substrate for the transformation of olfactory inputs to locomotor activity

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Aquatic vertebrates such as lampreys utilize chemical senses during movements associated with predation, migration and spawning. We have identified a neural substrate that allows for the transformation of an olfactory input into a locomotor output. Olfactory sensory input is transmitted to projection neurons (PNs) located in the medial region of the olfactory bulb. These project to the posterior tuberculum in the ventral diencephalon, where signals are directed to the mesencephalic locomotor region to reach reticulospinal cells in the lower brainstem (the command neurons for locomotion). Activation along this olfactory-motor pathway generates swimming behavior. Sensory neurons located in the accessory olfactory organ project axons only into the medial region of the olfactory bulb containing the PNs for this olfactory-locomotor substrate; although infrequent olfactory sensory neurons in the main olfactory epithelium also project into this medial bulbar territory. Amino acid, bile acid and pheromone odorants elicit monophasic and multiphasic local field potentials as well as multi-unit responses in this medial region. The dendrites and somata of these medial PNs are confined to glomerular neuropil, and are not observed in any of the deeper layers. The organization and responsiveness of this olfactory –locomotor substrate suggests that it is unique compared to the rest of the olfactory system, in terms of input, output, neuroanatomy and chemical responsiveness.

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Poster session I Poster #421

Luminally applied free amino acids modulate gastric exocrine and endocrine secretion in rats

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Amino acid sensing molecules, such as mGluRs and CaSRs, have been recently identified in membranes of endocrine and exocrine cells of gastric glands. Stimulation of these receptors by free luminal amino acids could affect gastric secretion directly or via vagal nerve circuits. In the present study we assessed influences of the intragastric application of amino acids on the release of acid, bicarbonate, pepsinogen, gastrin and somatostatin (SST). Experiments with anesthetized Sprague Dawley rats were approved by ACUC of the Pavlov Institute of Physiology. The pylorus-ligated stomach was luminally perfused *in situ* with saline or isotonic solutions of amino acids at pH 4.0. Exocrine secretion was measured conventionally in the gastric effluent. Blood level of hormones was assessed by ELISA in samples withdrawn from the portal vein. Luminal perfusion for 40 min with monosodium glutamate (MSG, 50 mmol/L), L-Phe (50 mmol/L), or the mixture of 17 amino acids lacking glutamate (Elental diet ®, 10% solution) stimulated output of pepsinogen, but did not affect secretion of H^+ and HCO_3^-. Infusion of Elental diet, but not MSG or L-Phe, elevated portal blood level of gastrin 34 and SST. Supplementation of MSG to Elental diet caused inhibition of SST release. In conclusion, free dietary amino acids markedly modulate gastric secretion of pepsinogen, gastrin and somatostatin *in vivo*. The obtained results are in good agreement with earlier *in vitro* findings that mGluRs are expressed in pepsinogen-secreting chief cells and SST- secreting D cells, and stimulation Gi-protein-coupled mGluRs results in inhibition of SST release.

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Poster session I Poster #191

Disruption of olfactory sensory neuron maturation by the absence of adenylate cyclase 3.

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The mammalian olfactory epithelium exhibits a distinct stratified organization, where the continuously generated olfactory sensory neurons (OSNs) are differentially positioned at specific developmental stages and readily identified with specific markers. It provides an opportunity to study what factors are critical for establishing the identity of a neuron. As OSNs mature, they stop expressing GAP43 and begin to express OMP, as well as the key components of odor-evoked signaling cascade such as AC3 and CNGA2. We have explored the roles of these signaling molecules in the maturation of OSNs in mice. Although odor-evoked electrical activity is abolished in the CNGA2-/- mice, at P20 OSN maturation appeared normal. In stark contrast, in the AC3-/- mice in which both odor-evoked biochemical and electrical activity are blocked, at P20 OMP positive mature OSNs were greatly reduced, while GAP43 positive immature OSNs and neurogenesis were drastically increased. Our further analysis showed that the vast majority of immature OSNs failed to reach maturity and eventually died via a Caspase3 dependent apoptotic pathway. In addition, in the olfactory bulb of the AC3-/- mice, numerous active Caspase3 positive OSN axons were detected, suggesting a possibility that although OSN axons in the AC3-/- mice were able to reach the bulb, their failure to form synapses there leads to their untimely death. To study the synaptic formation of OSN axons in the bulb, we developed a postnatal electroporation technique to visualize the distribution of synaptic vesicle proteins in the arbors of single OSN axons. We observed in the AC3-/- mice that OSN axonal arbors were less elaborated, synapse number was reduced and synapse distribution was altered. However, no such changes were seen in the CNGA2-/- mice. Together our results suggest that odor-evoked biochemical activity (cAMP levels) rather than electrical activity (action potentials) is critically involved in the maturation of mammalian OSNs.

Poster session I Poster #107

Optogenetic analysis of connectivity and circuit dynamics in the zebrafish homolog of olfactory cortex

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The small size and relatively low number of neurons make zebrafish an attractive model system for the structural and functional analysis of vertebrate neural circuits. The dorsal posterior telencephalon (Dp) of zebrafish is the homolog of olfactory cortex and receives direct excitatory input from mitral cells in the olfactory bulb. Previous studies showed that Dp neurons integrate information conveyed by distributed processing channels in the olfactory bulb and suggest that olfactory cortex functions as an auto-associative memory network. According to these hypotheses, intra-cortical excitatory and inhibitory connections play important roles in controlling cortical response patterns. We study functional interactions between different types of neurons in Dp using a combination of optogenetics and electrophysiology. We developed an electroporation approach to introduce optogenetic probes and genetically encoded calcium indicators into sparse sets of neurons in Dp of adult zebrafish. Upon optical stimulation of neurons expressing channelrhodopsin-2 (ChR2), we recorded synaptic responses of ChR2-negative neurons by whole-cell patch clamping in an ex-vivo preparation of the intact brain. Preliminary data show that synaptic responses are complex, including not only fast excitatory or fast inhibitory post-synaptic currents, but also slow and delayed currents that are likely to result from multi-synaptic network interactions.

Poster session II Poster #262

The influence of short–term memory on classical discrimination and cued identification olfactory tasks.

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Amongst the various techniques to assess olfactory functions, discrimination and cued identification are those potentially prone to the influence of odour memory. In the discrimination task, the participants are requested to detect the differing-smelling substance among three (see e.g., Hummel at al., 1997), without having the possibility to re-smell the substances. The time dynamics of the task may generate an un-intended memory component as the memory traces of the substance may decline before a response is given. Analogously, in cued identification tasks where the participants are requested to select a label among three-four to identify a presented odorant (Hummel et al. 1997; Doty et al, 1984) the interval between smelling and reading the labels (although very short) may affect the results. In this study, we have examined five groups of 12 participants each [i.e., Adolescents (age range 11-13 years), Young (age range 18-24) Middle-aged (age range 36-41) Elderly 1 (age range 65-71) Elderly 2 (over 72)] on the following tasks: classical discrimination, repeated discrimination, classical cued identification and simultaneous cued identification. The participants were let free to smell more than once the odorants on the repeated discrimination task and they were read aloud the labels while smelling on the simultaneous identification task. Only in the Elderly 2 group was performance on both classical discrimination and identification tasks worse than on the repeated discrimination and simultaneous identification tasks. This may be attributable to a short-term odour memory deficit. The present results suggest to be cautious in the clinical and experimental settings in the use of classical identification and discrimination tasks at least where (as in ageing and in various pathological condition) memory loss is a common feature.

Symposium 17 "Toward a genetic basis for human olfaction" Tuesday 26 June

Congenital general anosmia caused by an inherited sodium channelopathy

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Studies of mendelian heritable disorders and their genotype-phenotype relationships have provided important insights into complex functions of our sensory systems under normal and pathological states. These investigations led to rapid advances in our understanding of blindness, deafness, and pain disorders. However, progress in understanding the genetic basis of the human sense of smell has been slow. In the pain system, many of the heritable monogenic pain disorders have been mapped to mutations in genes encoding ion channels, leading to a growing list of channelopathy-associated human pain syndromes. One such ion channel that has been the focus of much recent attention is the voltage-gated and TTX-sensitive sodium channel Nav1.7, encoded by the gene *SCN9A*. Loss of function of this gene causes a congential inability to experience pain in humans. A significant advance in our understanding of olfaction came when it was recently shown that loss-of-function mutations in Nav1.7 not only affect pain perception but also eliminate odor perception in both mice and humans, leading to the discovery of the first ion channel gene essential for human olfaction. These findings also provide mechanistic insight into the critical role of Nav1.7 in axonal and synaptic signaling of olfactory sensory neurons and they are of special clinical relevance because Nav1.7 is a sodium channel that is currently being explored as a promising target for the pharmacotherapy of pain in humans. Taken together, these advances offer a functional understanding of a monogenic human disorder that is characterized by a loss of two major senses – nociception and smell – thus providing an unexpected mechanistic link between these two sensory modalities.

Author index (**bold**=presenting author)