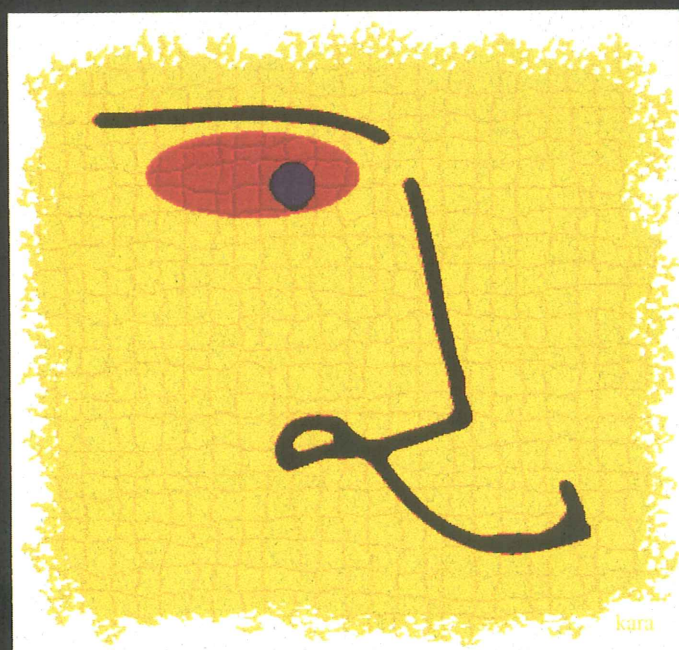


Abstracts

The Association for
Chemoreception Sciences
presents the
Sensory World of
Olfaction and Taste



ACheMS 2002

XXIVth Annual Meeting
April 24-28, 2002 • Sarasota, Florida

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1 Givaudan Lecture

Givaudan Lecture

REORGANIZATION OF SENSORY MAPS AFTER SENSORY LOSS IN DEVELOPING AND ADULT PRIMATES

Kaas J.H.¹ ¹*Department of Psychology, Vanderbilt University, Nashville, TN*

The brains of mammals are characterized by a number of orderly representations of sensory surfaces. The representations are subject to minor and major topographic modifications or reorganizations in both developing and mature brains. The modifications are most profound after large losses of sensory inputs, but they also occur as a result of changes in sensory experience and stimulation. The changes are mediated by a range of mechanisms, and they appear to be responsible for both adaptive changes in behavior and maladaptive and unwanted changes in perception and sensation. Such changes in the brain are not limited to the neocortex, but they are most easily studied there, and cortex may be capable of greater change. Plastic changes are not limited to sensory and motor representations, but they are most easily studied in these better understood parts of the brain.

2 Slide **Functional Consequences of Manipulating Chemosensory Systems****SIGNAL TRANSDUCTION OF UMAMI TASTE BY -GUSTDUCIN AND -TRANSDUCIN**

He W.¹, Margolskee R.F.², Damak S.¹ ¹*Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY;* ²*Howard Hughes Medical Institute, New York, NY*

The transduction events underlying umami taste, elicited by monosodium glutamate (MSG), are poorly understood. One G-protein coupled receptor, taste mGluR4, has been identified in taste cells and implicated in umami taste. To determine if -gustducin might be involved in umami responses, we carried out two bottle preference (TBP) tests comparing -gustducin knockout (KO) with wild-type mice. We found that -gustducin KO mice showed a diminished response to MSG. We hypothesized that the residual response of these mice might be carried by -transducin (also expressed in taste tissue). We carried out TBP tests comparing -gustducin and -transducin single and double KO and WT mice. Concentrations of MSG between 10 and 300 mM were preferred by WT and -transducin KO mice and less preferred by -gustducin KO mice, whereas the double KO animals were indifferent to these concentrations. IMP concentrations between 5 mM and 100 mM were preferred by WT and -transducin KO mice, whereas -gustducin KO and double KO mice were indifferent to the compound at these concentrations. With high concentrations of IMP or MSG (aversive to WT mice), there was little difference between the four groups. These data indicate that -transducin and -gustducin are involved in the transduction of umami preference. MSG signals appear to be transduced via both G proteins, whereas IMP signals are transduced only via -gustducin. Supported by NIH grants DC03055 and DC3155 (RFM), and DC04766 (SD). RFM is an Investigator of the Howard Hughes Medical Institute.

3 Slide **Functional Consequences of Manipulating Chemosensory Systems****EFFECT OF TRANSGENIC OVER-EXPRESSION OF NR2B ON OLFACTORY MEMORY PERFORMANCE IN THE MOUSE**

White T.L.¹, Youngentob S.L.¹ ¹*Neuroscience & Physiology, State University of New York Health Science Center at Syracuse, Syracuse, NY*

The olfactory and hippocampal systems of rodents are closely interconnected, so it is perhaps not surprising to note that mice have an exceptional capacity for olfactory learning and memory (Eichenbaum, 1998). The NMDA receptor in the hippocampus is thought to modulate learning and memory and the NR2B subunit is particularly relevant to this process. Transgenic mice (Tg) with over-expressed NR2B receptors demonstrate superior retention of spatial and visual information over wild type (WT) mice (Tang et al., 1999). Since the salient sensory system for rodents is olfaction, the transgenic mouse offers an appropriate model to ask whether over-expression of the NR2B subunit also alters olfactory learning and memory. In the first experiment, 12 mice (6 Tg/6 WT) completed both an olfactory and a visual Novel Item Recognition Task. After a 1-day retention interval, Tg mice remembered the visual objects significantly better than the WT mice ($t=2.39$, $p=0.02$), thus replicating earlier reports (Tang et al., 1999). In contrast, there was no difference in olfactory memory performance between the two groups ($t=0.95$, $p=0.18$). The second experiment examined the rate of acquisition on a go/no-go two-odor discrimination task (6 Tg/ 6 WT). The results showed no difference in the rate of acquisition between the groups ($t=0.45$, $p=0.33$). Taken together, these data suggest that, unlike spatial and visual information, olfactory learning and memory is not enhanced by the over-expression of the NR2B subunit. Supported in part by NIH grant number 5R01DC0447403

4 Slide **Functional Consequences of Manipulating Chemosensory Systems****ODOR DETECTION AND ODOR DISCRIMINATION IN BILATERALLY OLFACTORY BULBECTOMIZED RATS**

Slotnick B.¹, Pickett E.¹, Cockerham R.¹ ¹*Psychology, American University, Washington, DC*

After removal of 1 olfactory bulb in P2 rats, the homolateral forebrain grows forward to fill the resulting cavity and axons from maturing olfactory sensory neurons extend through the cribriform plate and terminate in the overlying cortical tissue. We assessed olfaction in such cases by training on odor detection and discrimination tasks at P70 and then retesting animals after removing the remaining olfactory bulb. Connections between the epithelium and forebrain were documented using anterograde transport of horseradish peroxidase (HRP) applied to the epithelium. In all cases, both olfactory bulbs were completely absent and the forebrain ipsilateral to the neonatal bulb removal contained HRP reaction product characteristic of axon bundles and clusters of axons that formed glomerular-like structures in frontal pole neocortex or in the olfactory peduncle. In some but not all cases with rich input to olfactory peduncle, rats were able to detect a variety of odors and discriminate between acetic acid and propionic acid and between the enantiomers of carvone. Performance in these cases was at or near that of controls (those with 1 intact olfactory bulb). Rats with input to only neocortex were anosmic as were those with extensive damage to the olfactory peduncle in the neonatal operated hemisphere.

5 Slide Functional Consequences of Manipulating Chemosensory Systems

CNGA4 NULL MICE EXHIBIT BEHAVIORAL DEFECTS IN ODOR THRESHOLD SENSITIVITY AND ADAPTATION

Kelliher K.R.¹, Ziesmann J.¹, Munger S.D.¹, Reed R.R.², Zufall F.¹

¹Dept. of Anatomy and Neurobiology, University of Maryland at Baltimore, Baltimore, MD; ²HHMI and Dept. of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD

An important role of adaptation in sensory systems is to prevent saturation of the transduction machinery. We have previously reported that, in the absence of the modulatory cyclic nucleotide-gated channel subunit CNGA4, olfactory receptor neuron responses display a decreased odor sensitivity and a pronounced defect in rapid odor adaptation (Munger et al., Science 2001, 294:2172). We tested whether these defects translate into changes in odor detection thresholds in CNGA4 null mice by employing an operant task using a commercially available olfactometer. Null mice exhibit an increase, up to 100-fold, in detection threshold for single odorants when compared with wildtype mice, indicating that the presence of CNGA4 in the native channel is essential for overall sensitivity. We then tested the ability of mice to detect a brief odor stimulus while being exposed to a continuous background odor. When a high concentration of background odor is added to the testing chamber, both +/+ and -/- mice show an initial drop in their ability to detect the test odor. However, +/+ mice quickly adapt, returning to normal detection performance while -/- mice fail to recover until the background odor is removed. These results demonstrate the importance of the CNGA4 subunit for rapid odor adaptation at the behavioral level, indicating the critical role of channel desensitization in odor detection. Supported by NINDS and NIDCD (K.R.K., S.D.M., R.R.R. & F.Z.).

6 Slide Functional Consequences of Manipulating Chemosensory Systems

VANASO IS A QUANTITATIVE TRAIT LOCUS FOR DROSOPHILA OLFACTORY BEHAVIOR

Fanara J.J.¹, Robinson K.O.¹, Mackay T.F.¹, Anholt R.R.¹ ¹W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC

Odor-guided behavior is a quantitative trait, with phenotypic variation due to multiple segregating loci (QTL). We used a stepwise gene discovery strategy to identify a gene that contributes to sex-specific quantitative variation in olfactory behavior. First, we quantified avoidance responses to benzaldehyde in 98 recombinant inbred lines derived from two homozygous strains of *D. melanogaster* and found a single QTL at cytological map position 61A - 66B. Next, deficiency complementation mapping resolved this region into one female- and one male-specific QTL, located at 62B1-8 and 66B2-8, respectively. Finally, we conducted quantitative complementation tests to all available mutations of candidate genes in these regions. The female-specific QTL failed to complement a P-element insertional mutation, *l(3)04276*. The P-element insertion was in the intron of a novel gene, *Vanaso*, which contains a putative guanylate binding protein domain and has a human homologue. Sequence analyses show that *Vanaso* contains multiple polymorphisms (SNPs and indels). Since *Vanaso* contributes to variation in olfactory behavior, it should be expressed in tissues that mediate recognition or processing of chemosensory information. Indeed, *in situ* hybridization reveals that *Vanaso* is expressed exclusively in the third antennal segment, but not in other chemosensory organs or elsewhere in the fly brain. This suggests that *Vanaso* plays a role in peripheral chemoreception rather than in central integration of olfactory information. Supported by NIH grant GM59469, the W. M. Keck Foundation, and a CONICET fellowship (to JJF).

7 Slide Functional Consequences of Manipulating Chemosensory Systems

REGULATION OF INSECT OLFACTION BY SENSORY ARRESTINS

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¹Department of Biological Sciences, Vanderbilt University, Nashville, TN; ²Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT

Olfaction exerts the primary influence over host choice in many insects, including the malaria vector mosquito, *Anopheles gambiae*. In an effort to characterize molecular components underlying chemosensory pathways in *A. gambiae*, we have identified a novel sensory arrestin, *AgArr1*. *AgARR1* has been localized to both olfactory and visual systems in *A. gambiae* and exhibits significant homology to arrestins from *Drosophila melanogaster*. Examination of *D. melanogaster* arrestins, previously thought to be primarily visual, has demonstrated that these genes (*DmArr1* and *DmArr2*) also show dual sensory system expression, suggesting that visual arrestins are involved in the regulation of olfactory signaling in these insects. To test this hypothesis, we have used EAGs and EPGs to examine olfactory responses in several *Drosophila* arrestin mutants. Initial results showed that *DmArr1* or *DmArr2* mutant alleles are associated with a decreased response amplitude indicating that both genes have olfactory roles. Current studies seeking to expand this analysis and to examine impacts on olfactory desensitization will be discussed along with assays for behavioral phenotypes. Furthermore, transgenic studies are underway to test the ability of *AgArr1* to rescue olfactory phenotypes associated with *D. melanogaster* arrestin mutations. These studies may provide support for the hypothesis that *AgArr1* functions similarly in mosquito olfactory pathways. Ultimately, this research could lead to novel control approaches to impede the spread of malaria and other insect-borne diseases.

8 Slide Functional Consequences of Manipulating Chemosensory Systems

GENETIC MANIPULATION OF ODOR RECEPTORS IN DROSOPHILA

Shiraiwa T.¹, Van Der Goes Van Naters W.¹, Lessing D.¹, Warr C.², De Bruyne M.³, Carlson J.R.¹ ¹Molecular, Cellular and Developmental Biology, Yale Univ., New Haven, CT; ²School of Biol. Sci., Monash Univ., Clayton, VIC, Australia; ³Institut für Neurobiologie, Freie Univ., Berlin, Germany

We have genetically manipulated odor receptor genes in order to examine their functions *in vivo* and to integrate the molecular and physiological maps of the olfactory system. We have isolated deletion mutations of the *Or71a* gene, which is expressed in the maxillary palp. The maxillary palp is an olfactory organ that contains 6 well-characterized functional classes of ORNs, combined according to a strict pairing rule into 3 types of sensilla. Thus the pb1A and pb1B neurons are paired in the type pb1 sensillum; pb2A and pb2B are paired in the type pb2; pb3A and pb3B are paired in pb3. Mutants of *Or71a* show a reduced electropalpogram response to 4-methylphenol, an odor to which the pb1B neuron is narrowly tuned. Single-unit recordings from pb1 in the *Or71a* mutants show a loss of sensitivity to 4-methylphenol. Surprisingly, we find no activity-even spontaneous activity-of the pb1B neuron; one possible interpretation is that the *Or71a* is required for normal development of the pb1B neuron. The defects are rescued by a wild-type *Or71a* transgene. The other ORNs in the mutant appear normal. To map receptors onto the other physiological classes of neurons in the maxillary palp, we have used the promoters of *Or* genes to drive expression of GFP, and then recorded from green sensilla. Initial results suggest that pb1A, which is paired with pb1B and which exhibits a broader odor specificity, expresses the *Or85e* receptor. Supported by NIH grants DC 02174 and DC 04729.

9 Symposium Sensory Coding in the Molecular Era: The Neural Representation of Gustatory, Olfactory, and Somatosensory Stimuli

INTRODUCTION: SENSORY CODING IN THE MOLECULAR ERA: THE NEURAL REPRESENTATION OF GUSTATORY, OLFACTORY AND SOMATOSENSORY STIMULI.

Travers S.P.¹ ¹*Oral Biology, Ohio State University, Columbus, OH*

This symposium will explore how stimulus features in the somatosensory, gustatory, and olfactory realms are represented by neural mechanisms. An important theme is the specialization of receptors for discrete functions, but equally important, how individual neural elements cooperate to allow sensory discriminations. In the somatosensory system, different types of receptor are often activated by the same physical stimulus. However, careful analysis of the relationships between response properties and behavior suggests that specialized receptor types use precise mechanisms to code for specific types of discrimination. However, it is equally apparent that somatosensory discrimination requires cooperation between large neuronal ensembles. Theories of gustatory coding have long been dominated by contrasting options about whether taste quality is coded by discrete classes of neurons conveying information about a particular quality, or by neuronal assemblies that participate in more than one type of message. Theories of olfactory coding usually presuppose either an ensemble code or a labeled line code, and current investigations focus on understanding the coding strategies that the system uses in response to a variety of odorant molecules. These themes will be reevaluated and more fully developed, in light of recent data from molecular and cellular studies of transduction, in vivo peripheral and central neurophysiology, and imaging studies.

10 Symposium Sensory Coding in the Molecular Era: The Neural Representation of Gustatory, Olfactory, and Somatosensory Stimuli

A COMBINED PSYCHOPHYSICAL AND NEUROPHYSIOLOGICAL STUDY OF THE NEURAL MECHANISMS OF TACTILE TEXTURE PERCEPTION

Johnson K.O.¹, Hsiao S.S.¹, Yoshioka T.¹ ¹*Neuroscience (SOM), Johns Hopkins University, Baltimore, MD*

The subject of this talk is a study of the neural coding mechanisms underlying texture perception. Subjects provided roughness magnitude estimates in 4 experiments with controlled surfaces. The same surfaces were used in neurophysiological studies in monkeys and humans to get statistically accurate estimates of the entire population response evoked by each surface. The experimental design adhered rigorously to the scientific method. All plausible neural coding hypotheses (>20) were formulated (intensive, temporal, and spatial coding mechanisms within and between four receptor populations). The object of each experiment was falsification, not what works best. The test of each hypothesis was consistency: a putative neural code was rejected only when there was no consistent relationship between its neural measure and subjects' magnitude estimates. There were four major results: (1) All but one hypothesis failed completely in one or more experiments; the single survivor - perceived roughness depends on the mean absolute difference in firing rates between afferents with receptive field centers separated by 2-3 mm - was highly consistent in all experiments ($r > 0.97$). (2) Subjects' magnitude estimates were related linearly to this measure although nothing in the analysis was predisposed to linearity. (3) Separate psychophysical experiments imply that the measure is computed by neurons with receptive fields confined to a single finger. (4) Later experiments showed that neurons in area 3b, which have RFs confined to a single finger, compute this measure.

11 Symposium Sensory Coding in the Molecular Era: The Neural Representation of Gustatory, Olfactory, and Somatosensory Stimuli

SIMILARITIES BETWEEN THE DYNAMIC AND DISTRIBUTED NATURE OF NEURONAL ENSEMBLE ACTIVITY IN THE PRIMARY SOMATOSENSORY AND GUSTATORY CORTICES

Nicolelis M.A.¹ ¹*Department of Neurobiology, Duke University Medical Center, Durham, NC*

In this talk I will describe recent findings, obtained through chronic and simultaneous multi-electrode recordings in behaving rats, which demonstrate the relevance of the temporal component in cortical representations of both tactile and gustatory information. I will also present evidence suggesting that purely tactile inputs from the perioral region, which are relayed through the primary somatosensory (SI) cortex, contribute to the definition of tastant responses in the primary gustatory (GI) cortex. These two findings will serve as the basis for a comparison of the type of neural ensemble interactions observed in both SI and GI in behaving rats.

12 Symposium Sensory Coding in the Molecular Era: The Neural Representation of Gustatory, Olfactory, and Somatosensory Stimuli

INDIVIDUAL NEURONS AND TASTE QUALITY CODING

Smith D.V.¹ ¹*Anatomy & Neurobiology, University of Maryland, Baltimore, Baltimore, MD*

From the earliest electrophysiology on the mammalian gustatory system, investigators have recognized that individual cells are not specifically tuned to stimuli of one taste quality. This multiple sensitivity has led to a long debate over the role of individual neurons in the code for quality, with one approach advocating a population code and another a labeled-line representation. Recent molecular studies have reawakened interest in this debate, primarily because it is easy to think about molecular mechanisms one cell at a time and to, therefore, imagine a "sweet" or a "bitter" cell based on the expression of a particular receptor protein. However, electrophysiological studies, from receptor cells to central neurons, continue to demonstrate the multiple sensitivity of individual gustatory neurons, calling into question the role of any one of them in taste quality coding. Classes of gustatory neurons that might theoretically comprise labeled lines are clearly critical in defining the similarities and differences among taste stimuli. Whether these cell types alone, however, are both necessary and sufficient to represent a particular quality is the essential question and one that may not be answerable at present. Considerations of the signal-to-noise ratio in the response of individual cells, their nonlinear concentration-response relationships, and the fact that more information can be conveyed by cell ensembles than by single neurons makes a population model the more attractive alternative to explain taste quality coding, although questions still remain about the relationship between the neural code and the analytic nature of the perception of taste mixtures. Supported by NIDCD DC00353 and DC00066.

13 Symposium Sensory Coding in the Molecular Era: The Neural Representation of Gustatory, Olfactory, and Somatosensory Stimuli

OLFACTORY CODING

Leon M.¹, Johnson B.A.¹ ¹*Neurobiology and Behavior, University of California, Irvine, Irvine, CA*

An olfactory code describes the transposition of odorant molecular information into a neural representation, and understanding such a code should include the ability to predict: an odorant molecule from its neural representation, the neural representation of novel odorant molecules, and the perception from odorant-evoked neural activity. To study an olfactory code, we exposed rats to a wide variety of carefully selected odorants and observed responses across the entire glomerular layer. Response patterns were mapped into a data array that allowed us to compare group response profiles. While some odorants appear to be processed in highly focal representations consistent with single-receptor coding, many odorants are coded by a combination of focal responses to specific molecular features. The size and intensity of the representation vary systematically and predictably with concentration, molecular structure and previous experience. Odorant-evoked activity is clustered within glomerular modules, which are domains responsive to either functional group or hydrocarbon structure. The modular responses are systematic enough and reliable enough to predict accurately odorant stimuli from the evoked glomerular response, as well as the glomerular response for novel odorants. Responses within a module can be organized systematically with respect to differences in odorant structure, suggesting that both an identity code and a spatial code are used in this system. Finally, odorant-evoked neural representations accurately predict odor perceptions in a way that reveals two olfactory processing modes. Supported by DC03545.

15 Poster

Ion Channels

USE OF REAL-TIME PCR TO QUANTITATE DIFFERENCES IN EXPRESSION OF DELAYED RECTIFYING POTASSIUM CHANNELS IN TASTE CELLS

Hansen D.R.¹, Kwon S.¹, Gilbertson T.A.¹ ¹*Biology, Utah State University, Logan, UT*

Delayed rectifying K (DRK) channels have been demonstrated to play a variety of roles in the taste transduction process including shaping tastant-induced action potentials and serving as a target both directly and indirectly in the transduction pathways of a variety of different classes of gustatory stimuli. There has been little or no direct evidence concerning the types and relative quantities of DRK channels expressed in mammalian taste receptor cells. We have used a multiplexed TaqMan assay with real-time polymerase chain reaction (PCR) to quantitate expression of DRK channels in rat taste buds relative to the housekeeping gene, GAPDH, which we have shown to be expressed at the same levels in the three types of lingual taste buds. Using this approach, we have found significant differences in expression of DRK channels in taste buds from the fungiform (FF), foliate (FO) and circumvallate (CV) papillae. In general, FO and CV taste buds express similar profiles of DRK channels, which are different from those expressed in FF taste buds. For example, compared to the CV and FO taste buds, FF express significantly greater amounts of Kv3.2 mRNA and less Kv2.1 and Kv3.1. The differences in expression of DRK channels and its implications for taste transduction will be discussed. Furthermore, the use of quantitative real-time PCR assays such as those described will prove useful for examining both developmental and regulatory changes in the expression of cellular elements involved in taste transduction pathways. *Support was provided by NIH DK55809 (TAG).*

14 Poster

Ion Channels

LIVE CELL IMAGING OF MG²⁺ INFLUX DURING THE BIOTIN OFF-RESPONSE IN PARAMECIUM

Bell W.E.¹, Green M.H.¹ ¹*Department of Biology, Virginia Military Institute, Lexington, VA*

Magnesium can contribute significantly to membrane excitation and ciliary reversal in *Paramecium*. Unlike most observed magnesium currents, I_{Mg} in *Paramecium* is specific for magnesium and is calcium impermeant (Preston, 1998, J. Membrane Biol. 164: 11-24). A mutant (*eccentric*) that is defective in I_{Mg} does not respond to the chemorepellant GTP when other ions, such as Na^+ are unavailable, suggesting a role in chemoresponse for magnesium. This GTP-induced current is thought to be Ca^{2+} dependent (Clark, et al., 1997, J. Membrane Biol. 157: 159-167). We have made a similar observation in behavioral assays with the chemoattractant biotin. With Mg^{2+} and Ca^{2+} as the only extracellular ions, *eccentric* loses the strong attractant response it displays when either K^+ or Na^+ are present. Initial electrophysiological measurements indicate that a Mg^{2+} current is activated after removal of biotin. These data suggest that an "off-response" could be the primary mediator of chemoattraction to biotin in *Paramecium*. We have developed a system for restraining live *Paramecium* so that they may be observed microscopically while their external environment is altered via perfusion. Cells loaded with the membrane-permeant Mg^{2+} sensitive fluorescent dye Magnesium Orange AM (Molecular Probes) were perfused with Mg^{2+} biotin which was then exchanged for a $MgCl$ solution. As $MgCl$ replaced the biotin solution in the chamber, a slow increase in Mg^{2+} was observed. Mg^{2+} levels returned to baseline after several minutes in the $MgCl$ solution. The increase in cytosolic Mg^{2+} levels during "off-response" is being investigated with other *Paramecium* attractants. This work was supported by VMI and the Jeffress Memorial Trust.

16 Poster

Ion Channels

EXPRESSION OF THE CLC FAMILY OF CHLORIDE CHANNELS IN TASTE CELLS

Rao S.¹, Hansen D.R.¹, Gilbertson T.A.¹ ¹*Biology, Utah State University, Logan, UT*

Recently we demonstrated the presence of volume-activated chloride channels in taste cells that are functionally similar to the swelling-activated Cl^- channel ($I_{Cl,swell}$) found in many cell types capable of osmoregulation. We hypothesize that these channels in taste cells may play roles in the gustatory response to water and the mechanism of regulatory volume decrease (RVD) following prolonged exposure to hypotonic solutions. To identify potential candidates for the chloride channels mediating hypoosmotic responses in taste cells, we have performed RT-PCR using probes against some members of the ClC family of chloride channels. Though the identity of the channels mediating the ubiquitous $I_{Cl,swell}$ remains uncertain, one candidate remains ClC-3. Using RT-PCR with probes against ClC-3 and ClC-2, an inwardly rectifying Cl^- channel, we have found that all three types of lingual taste buds express both types of Cl^- channels. Subsequent sequencing of the PCR products reveals these channels in taste cells to be highly homologous to those found in kidney. One or both of these channels may be important for gustatory processing. We are currently attempting to identify additional members of the ClC family in mammalian taste buds. *Support was provided by NIH DK55809 (TAG).*

THE CA2+-ACTIVATED CL CONDUCTANCE IN RAT OLFACTORY RECEPTOR CELLS

Reisert J.¹, Yau K.¹, Frings S.² ¹*Department of Neuroscience, Johns Hopkins School of Medicine, Howard Hughes Medical Institute, Baltimore, MD;* ²*Institut für Biologische Informationsverarbeitung I, FZ Juelich, Juelich, Germany*

Stimulation of olfactory receptor cells (ORCs) activates a G-protein coupled cascade, which leads to the opening of cyclic nucleotide-gated (CNG) channels and influx of Ca²⁺. This increase in Ca²⁺ gates a Ca²⁺-activated Cl conductance, which carries a substantial percentage of the odor-induced excitatory receptor current. We used membrane patches excised from the knob of rat ORCs to study the characteristics of this channel. When the cytoplasmic side of an inside-out patch was exposed to Ca²⁺ in symmetrical NMDG-Cl, a current was recorded that could be blocked by niflumic acid. Upon multiple exposures to Ca²⁺ this Cl current progressively decreased ('rundown') over a time scale of minutes, losing on average half its magnitude, while the cAMP-gated current remained constant. This reduction in current was not associated with a shift in Ca²⁺ sensitivity. Ca²⁺ sensitivity and inactivation kinetics were studied by exposing patches to a range of Ca²⁺ concentrations for 10 s. The Ca²⁺ concentration needed to half-activate the Cl current was 2.2 μM at V_m = -40 mV and 1.5 μM at +40 mV. Over the 10s Ca²⁺ stimulation, the current reversibly inactivated and was reduced to around 65 % of its initial peak value. This was not caused by ion depletion within the patch, but rather due to an intrinsic inactivation of the current. The permeability ratios of halides was measured to be P_{Cl}:P_{Br}:P_I = 0.1:1.2:1.3:4. Further experiments will address questions of channel regulation and the interplay of CNG and Ca²⁺-activated Cl channels.

IP3 RECEPTORS AND OLFACTORY RECEPTOR TRANSDUCTION

Liu G.¹, Badeau R.¹, Tanimura A.², Talamo B.¹ ¹*Neuroscience, Tufts U. School of Medicine, Boston, MA;* ²*Dental Pharmacology, U. of Hokkaido, Japan*

Previously we have shown that the intracellular Ca²⁺ increase induced by odorants in Odora cells transfected with the odorant receptor U131 depends on extracellular Ca²⁺. No intracellular Ca²⁺ is released. These responses are reversibly inhibited by PLC inhibitor, U73122, and IP3 receptor (IP3R) antagonist, Xestospongion C (Chem. Senses 26:1126,2001). We investigate the involvement of IP3Rs in mediating the odorant response by examining their expression and localization in Odora cells by immunoprecipitation, Western blot and cell surface biotinylation. All three types of IP3R are present in Odora cells as well as in rat olfactory epithelium. We directly labeled intact Odora cells with the membrane-impermeant biotinylation reagent, sulfo-NHS-biotin, and captured the biotinylated plasma membrane proteins with avidin beads. Using antibodies specific for each of the three IP3R subtypes, we provide strong evidence that a fraction of each of these subtypes is present on the plasma membrane of Odora cells. The percentage of IP3R on the cell surface varies among the different subtypes and with cell differentiation. About 10% of the total cellular IP3R type 1, type 2 or type 3 is on the cell surface in undifferentiated Odora cells. In differentiated cells, the fraction of IP3R type 2 on the plasma membrane is approximately doubled, while the fraction of type 1 and type 3 remains unchanged. These data suggest that besides the traditional role of IP3Rs on the endoplasmic reticulum, IP3Rs on the plasma membrane may play an important role in transducing odorant receptor signaling in Odora cells, possibly providing a pathway for Ca²⁺ entry.

EXPRESSION OF NA+/CA2+/(K+) EXCHANGERS IN OLFACTORY RECEPTOR NEURONS (ORNS)

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Characterization of the downstream events in olfactory transduction lags behind studies of the initial events of receptor-ligand-mediated activation of G-proteins and adenylyl cyclases, channel opening, and Ca²⁺ influx. However, mechanisms that terminate the response and reset intracellular Ca²⁺ levels to baseline are important to return ORN sensitivity in the overall transduction process. Recent reports (Reisert and Matthews, 2001) redirect attention to the latter events and demonstrate the role of Na⁺/Ca²⁺ exchange in terminating the transduction current. Curiously, manipulation of Na⁺/Ca²⁺ exchange efficiency by altering the extracellular sodium concentration induced effects similar to those seen in the OMP-null mouse. Thus, it is important to study the mechanism(s) by which elevated intracellular ORN Ca²⁺ is eliminated and returned to baseline. There are three Na⁺/Ca²⁺ exchanger genes (NCX1, 2, 3) and three Na⁺/Ca²⁺/K⁺ exchangers (NCKX1, 2, 3). Transcription of some of these genes is subject to differential splicing, generating multiple mRNAs. There is virtually no molecular information about which of the NCX and/or NCKX exchanger genes or mRNA splice variants are expressed by ORNs, the cellular localization of the corresponding proteins and how OMP might modulate the exchangers' activities. This prompted us to analyze NCX and NCKX gene expression in mouse olfactory tissue during development and in response to lesions. Supported in part by NIH DC03112 (FLM) and NIH HL62521 (DHS).

CALMODULIN-MEDIATED OLFACTORY ADAPTATION: THE ROLES OF CAMP-GATED CHANNEL SUBUNITS.

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Calcium (Ca²⁺) influx through Ca²⁺-permeable ion channels plays a pivotal role in a variety of neuronal signaling processes, and negative-feedback control of this influx by Ca²⁺ itself is often equally important for modulation of such signaling. Negative modulation by Ca²⁺ through calmodulin on cyclic nucleotide-gated (CNG) channels underlies the adaptation of olfactory receptor neurons to odorants. Previously we showed that in order for this feedback regulation to be both rapid and independent of the channels' open-state, three subunits, CNGA2, CNGA4 and CNGB1b must be part of the heteromeric channel complex. Thus, modulatory subunits are essential for calmodulin binding, despite the prevailing view that the machinery for calmodulin binding and modulation is present in the principal subunit, CNGA2. In order to distill the roles of individual subunits in mediating the rapid feedback modulation, and to determine which subunits function as the calmodulin effectors, we have undertaken a series of experiments using deletion mutants. In particular we have focused on the contribution of two calmodulin binding sites in CNGB1b and a third in CNGA2. Our results illustrate the importance of CNGB1b in the calmodulin-dependent adaptation of olfactory sensory neurons. Supported by: DFG, HHMI and Forschungszentrum Jülich.

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Ion Channels

MULTIPLE SUBTYPES OF VOLTAGE-GATED SODIUM CHANNELS ARE EXPRESSED BY MOUSE OLFACTORY SENSORY NEURONSFrenz C.T.¹, Dionne V.E.¹ ¹*Biology, Boston University, Boston, MA*

Voltage-gated sodium channels (VGSC) produce the action potentials that allow olfactory sensory neurons (OSNs) to tell the brain about odor. Nine isoforms of the structural subunit of VGSCs are known. These isoforms are expressed in different neural tissues, have subtly different physiological properties, can be differentially regulated, and may be expressed at different times during development. Sodium currents (INa) mediated by different VGSCs are differentially sensitive to tetrodotoxin (TTX). TTX-sensitive subtypes can be blocked by nanomolar concentrations of toxin, while TTX-insensitive subtypes require >1 μ M toxin. The relative sensitivity of INa to TTX was evaluated in mouse OSNs with whole cell patch recordings. Cells voltage-clamped in a TTX-free bath were exposed to 10nM and 1 μ M TTX. In 43% of cells 10nM TTX blocked 15-50% of INa, suggesting an EC50 near this concentration. In the majority of cells 10nM TTX had no effect while 1 μ M TTX blocked 55-90% of INa in all cells. These results suggest that OSNs express both TTX-sensitive and TTX-insensitive VGSCs. RT-PCR experiments confirmed the presence of mRNA for multiple isoforms of the main subunit of VGSCs in olfactory epithelial tissue. Four isoforms were detected: *Nav1.2*, *Nav1.3*, *Nav1.5* and *Nav1.6*. *Nav1.5* is a TTX-insensitive subtype, while the others are all TTX-sensitive. These results establish the presence of multiple subtypes of VGSCs in mouse OSNs and suggest they are distributed non-uniformly among the neurons. The physiological and molecular differences of these channels may have important roles in the development of OSNs or the regulation of neuronal excitability. Supported by NIH DC00256.

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Ion Channels

CALCIUM SENSITIVITY OF A SODIUM-ACTIVATED NON-SELECTIVE CATION CHANNEL IN LOBSTER OLFACTORY RECEPTOR NEURONSBobkov Y.V.¹, Ache B.W.¹ ¹*Whitney Laboratory, Center for Smell and Taste, and McKnight Brain Institute, University of Florida, Gainesville, FL*

Here we report that a novel Na⁺-activated non-selective cation channel in cultured lobster ORNs (Zhainazarov, et al., *J. Neurophysiol.* 79:1349, 1998) can also be activated by intracellular Ca²⁺. In the presence of 210 mM Na⁺, Ca²⁺ increases the open probability of the channel with a K_{1/2} of 490 nM and a Hill coefficient of 1.3. Ca²⁺ also increases the sensitivity of the channel to Na⁺, shifting the K_{1/2} for Na⁺ activation from 112 mM at 10 nM Ca²⁺ to 32 mM at 1-100 μ M Ca²⁺. Earlier, we reported that this channel can be inhibited by intracellular Ca²⁺. In a subset of cultured cells, channels with the same overall properties are insensitive to intracellular Ca²⁺. This apparent heterogeneity in Ca²⁺ sensitivity of the channel could be determined by the phosphorylation state of the channel. The nonspecific activator of protein phosphatase, protamine (1-5 μ g/ml), applied to the intracellular face of patches containing the channel irreversibly eliminated the sensitivity to Ca²⁺. The effect of protamine was blocked by okadaic acid (2 μ M), a nonspecific blocker of protein phosphatase, and restored by the catalytic subunit of PKA in the presence of 1 mM MgATP. Channels similarly activated by Ca²⁺ occur in the outer dendritic membrane of the cells *in situ*, suggesting the Ca²⁺ sensitivity of the channel serves a role in olfactory transduction. The physiological role of the Ca²⁺ sensitivity of the channel and the ability of phosphorylation to regulate that sensitivity are presently being explored *in situ*. Supported by a grant from the NIDCD.

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Ion Channels

MOLECULAR AND CELLULAR CHARACTERIZATION OF AN IH-CHANNEL FROM LOBSTER OLFACTORY RECEPTOR NEURONSMarx T.¹, Bobkov V.², Wetzel C.H.¹, Gisselmann G.¹, Ache B.W.², Hatt H.¹ ¹*Cell Physiology, Ruhr-University Bochum, Bochum, Germany;* ²*Whitney Laboratory, Center for Smell and Taste, and McKnight Brain Institute, University of Florida, Gainesville, FL*

Here we report a full-length ion channel activated by hyperpolarization and cyclic nucleotides (an HCN, or Ih, channel) cloned from lobster olfactory organ cDNA. Transfecting the coding sequence into HEK 293 cells gave a slowly activating, non-inactivating inward current under whole-cell voltage-clamp to hyperpolarizing voltage steps that reversed about -45 mV. The amplitude and activation rate of the current was hyperpolarization-dependent. cAMP or cGMP (1 mM) shifted the activation curve of the whole-cell current to less negative potentials in a concentration-dependent manner some 40 mV to within the normal resting potential range, accelerated the kinetics of channel opening, and increased the deactivation time of the channel. Under physiological conditions, hyperpolarization elicited an inward cation current in cultured lobster ORNs that reversed at -30 \pm 9 mV. cAMP (1mM) shifted the half-maximal activation of the current from -110 \pm 14 mV to -81 \pm 9 mV. Immunoreactivity to an antibody raised against the sequence occurred in the olfactory sensilla, as well as in the olfactory organ itself, muscle, brain, eye, and hepatopancreas. The physiological properties of the channel and its presence in the transduction zone suggest this otherwise ubiquitous channel may function in olfactory transduction in lobster ORNs. Supported by grants from the DFG, the NIDCD, and the A. von Humboldt Foundation.

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Development of the Olfactory System

OVEREXPRESSION OF XDLL3 AND XDLL4 RESULTS IN SIMILAR MORPHOLOGICAL CHANGE IN XENOPUS LAEVISCox J.T.¹, Burd G.D.¹ ¹*Molecular and Cellular Biology, University of Arizona, Tucson, AZ*

Distal-less genes are transcription factors involved in patterning early embryonic tissues. Two *Distal-less* genes in *Xenopus laevis*, *Xdll3* and *Xdll4* (homologs of murine *Dlx5*), are expressed in the olfactory bulb, ventral forebrain, branchial arches, cranial neural crest, and cement gland. *Xdll3*, but not *Xdll4*, is expressed in the olfactory and otic placodes, while *Xdll4*, but not *Xdll3*, is expressed in the eye. Given the different expression patterns, we hypothesized that overexpression of these genes would produce different developmental effects. To test this hypothesis, we injected 125 pg of *Xdll3* or 25 pg of *Xdll4* mRNA into one cell of a two-cell staged embryo. Morphology was analyzed in young larva. *Xdll4* and *Xdll3* overexpression results in an apparent reduction or absence of the eye and in malformed head structures, presumably due to effects on the neural crest and branchial arch derivatives. In addition, in some *Xdll3* and *Xdll4* animals, the nasal capsule and the olfactory bulb appeared to be distorted. These phenotypes resemble those described in the *Dlx5* knockout mouse (Depew et al., 1999). In addition, overexpression of transcription factors *in vitro* can suppress transcription of target genes (Czerny and Busslinger, 1995). Since the DNA binding domains of both *Xdll3* and *Xdll4* are nearly identical, we hypothesize that both genes interact with the same downstream target genes and that overexpression distorts development by acting to suppress transcription of unknown genes in the eye, branchial arch, neural crest, and possibly the olfactory system. Supported by NINDS #NS37147.

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REPRESENTATIONAL DIFFERENCE ANALYSIS OF METAMORPHIC CLIMAX VS. PRE-METAMORPHIC XENOPUS LAEVIS NOSES

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Forward and reverse representational difference analysis (RDA) was used to identify either enriched or suppressed mRNAs during metamorphic climax in the noses of the African clawed frog, *Xenopus laevis*. The goal was to identify genes involved in regulating the plasticity in the principle cavity and stimulating the formation and development of the middle cavity of the olfactory system at metamorphosis. Also, to begin to identify genes in the olfactory system which are regulated, directly and indirectly, by thyroid hormone. Following three rounds of subtraction, the forward RDA yielded 155 up-regulated difference products, and the reverse RDA yielded 19 down-regulated products. 10 random up-regulated clones were sequenced. Blast comparisons identified 4 *Xenopus* vitellogenin gene fragments, alcohol dehydrogenase class IV, *Xenopus* lens specific gene, and 4 non-specific matches. Sequencing and BLAST of 6 random down-regulated clones identified creatine kinase, alpha cardiac actin, cardiac myosin heavy chain, 2 myosin light chains and a sequence similar to ribosomal protein L19. There is precedence in *Xenopus* for a single gene to have multi-functional, multi-tissue specific and/or stage specific roles during development, metamorphosis and maintenance. Therefore, all results warrant investigation. High throughput sequencing of all 174 difference products is in progress. The clones obtained from both RDAs, along with appropriate controls, will be used to create cDNA microarrays. The arrays will be probed with stage specific mRNA to determine developmental expression of these genes. Support: NIDCD #DC03905

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IMMUNO-LOCALIZATION OF PACAP IN THE DEVELOPING AND MATURE RODENT VNO

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Recently, the role of amidated neuropeptides, like PACAP (pituitary adenylate cyclase activating peptide) has been investigated in the developing and mature peripheral olfactory system (1,2). This system is particularly interesting because of its ability to generate new receptor neurons throughout life. However, this ability is shared by the vomeronasal system, where pheromone-detecting receptor neurons also are continually generated within the neuroepithelium. Previously we showed that antibodies against PACAP₃₈ were immuno-localized to elements of the olfactory epithelium during development and in the adult (2). Here, we are continuing these studies in the developing and mature vomeronasal system, including the vomeronasal organ (VNO) and its target, the accessory olfactory bulb (AOB). In our preliminary work, PACAP₃₈ has not been detected in the neuroepithelium or the respiratory epithelium of the postnatal mouse VNO. However, we did see significant labeling in presumed nerve fibers encircling the vomeronasal vein, known to be innervated by sympathetic fibers as a component of the vascular pump, and in components of the AOB. We are continuing a thorough investigation of PACAP₃₈ immuno-localization of the fetal through mature vomeronasal system, as an initial step towards understanding the role of PACAP in this distinctive pheromone-processing complex. 1. Hansel et al. (2001) J. Neurosci. 21(13):4625. 2. Lucero et al. (2001) Chem. Sens. 26(8):1090. Supported by ISU Univ. Res. Comm. grant #681-145-01 to EWJ; NIH NIDCD grant #DC02994 to MTL

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LOCALIZATION OF ALDH 1 IN RAT OLFACTORY GLIA AND EFFECTS OF VITAMIN A-DEFICIENCY ON ITS EXPRESSION

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Retinoic acid (RA) is a transcription factor that affects cell proliferation and cell differentiation. The source of RA in mammalian systems has not been firmly established, but available evidence indicates it may be synthesized locally by a two step process catalyzed by independent alcohol and aldehyde dehydrogenases that promote the conversion of the alcoholic functional group of retinol (taken up from the bloodstream), first, to an aldehyde, and then to carboxylic acid. Aldehyde dehydrogenase Type I (ALDH I) shows substrate preference for retinaldehyde in vitro and, as such, is a candidate enzyme for local production of RA in vivo. It is reported here that protein recognized by an antibody directed against rat ALDH 1 is localized in sustentacular cells (supporting glia) in the olfactory neuroepithelium and in ensheathing cells (glia surrounding axons) and Bowman's Gland cells in the lamina propria underlying the olfactory neuroepithelium of vitamin A sufficient postnatal rats. The expression levels of ALDH I mRNA and protein are upregulated in olfactory tissue from vitamin A deficient rats relative to those of vitamin A-sufficient controls. The strongest expression of ALDH I in vitamin A-deficient tissue appears to be in Bowman's Glands. The results suggest that ALDH 1 is involved in retinoid metabolism in postnatal rat olfactory tissue in vivo. Supported by NIH/NIDCD # 1 KO2 DC 180-01 and NIH/NIGMS/MBRS # 5 S06 GM08092

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GROWING OLFACTORY RECEPTOR AXONS FROM MANDUCA SEXTA DISPLAY DIFFERENT INTERACTIONS WITH CENTRAL AND PERIPHERAL GLIA IN VITRO.

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During the development of vertebrate and invertebrate olfactory systems, axons of olfactory receptor cells (ORCs) encounter multiple types of glial cells located along their pathway. In the moth *Manduca sexta*, centrally derived glia in the sorting zone (SZ) reorganize ORC axons into fascicles destined to terminate in particular glomeruli, while glia in the antennal lobe (AL) facilitate glomerulus formation. Peripheral glia arise in the antenna and migrate down the antennal nerve (AN) days after the first axons have reached the AL. To ask how glia influence pathfinding decisions of ORC axons, we are studying *in vitro* interactions between ORC growth cones and glial cells isolated from the antennal system. Despite their different effects *in vivo*, glia from the SZ and the AL evoke similar contact-dependent changes in ORC growth-cone complexity and motility in culture, as visualized in time-lapse recordings: growth cones often stop advancing and form large, lamellar processes which persist for hours. In contrast, contact with AN glia leads not to arrest of growth, but to growth-cone advancement along glial processes, and effects on growth-cone complexity are only transient. Moreover, AN glia organize into multicellular chains along the axonal processes. Thus, centrally derived glial cells have effects that might enhance growth-cone exploration, changes in trajectory or fasciculation, and continued growth, whereas AN glia may serve simply as permissive struts for late-growing ORC axons. (Supported by NIH DC04598)

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A ROLE FOR EPH/EPHRIN SIGNALING IN DEVELOPING OLFACTORY AXONS

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Eph-family receptor tyrosine kinases and their ligand ephrins have been shown to play important roles in axon guidance. In the current study, we explore the possibility that Eph/ephrin signaling is involved in the guidance, sorting, and/or targeting of olfactory receptor cell (ORC) axons in the moth, *Manduca sexta*. Messenger RNA levels of both *Manduca* Eph (MsEph) and *Manduca* ephrin (MsEphrin) are upregulated during the time when ORC axons grow into the CNS. Ephrin-Fc, a recombinant protein in which the extracellular domain of MsEphrin is fused to IgG Fc, binds to and induces tyrosine phosphorylation of MsEph expressed in HEK cells, suggesting MsEphrin's function as a ligand. The receptor(s) and the ligand(s) detected by Ephrin-Fc and Eph-Fc, respectively, are localized on ORC axon terminals in a subset of glomeruli during their formation, disappearing after their establishment. The distribution of the receptor- and ligand-positive glomeruli appears to be complementary to each other among identifiable glomeruli. These spatial and temporal expression patterns suggest that MsEph and MsEphrin are involved in ORC axon targeting. We are examining the consequence of Eph/ephrin signaling in ORC axons using primary cultures of olfactory epithelium. Treatment with Eph-Fc or Ephrin-Fc dose-dependently inhibits neurite outgrowth from antenna explants, suggesting that endogenous MsEph/MsEphrin interactions are necessary for optimal axon growth. These results show that MsEph and MsEphrin are expressed in the developing ORC axons and that disruptions of their interactions have effects on axon growth. Supported by Flinn Foundation and Charles E. Culpeper 99-294.

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DIFFERENCES IN LECTIN-BINDING CONSTITUENTS ON OLFACTORY RECEPTOR AXONS PROJECTING TO ORDINARY AND SEXUALLY DIMORPHIC GLOMERULI IN MALE AND FEMALE MANDUCA SEXTA.

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Heterogeneous molecules on the axons of olfactory receptor cells (ORCs) may be key in guidance of the axons to their correct glomerular targets in both vertebrates and invertebrates. The antennal lobe (AL) of *Manduca sexta* contains several glomeruli that are sexually dimorphic, the macroglomerular complex (MGC) in the male and the large female glomeruli (LFGs) in the female. During metamorphosis in the male, ORC axons uniformly bind wheat-germ agglutinin (WGA). Following eclosion only those axons projecting to the MGC continue to bind WGA. Treatment of vibratome sections of male pupal ALs with protein N-glycanase F (PNGase F) virtually eliminates WGA binding by axons projecting to the "ordinary" glomeruli but not by those projecting to the MGC. Adult AL sections treated with PNGase F exhibit no decrease in WGA binding by MGC axons. In females, axons targeting all glomeruli continue to bind WGA in the adult and PNGase F treatment of pupal AL sections produces little or no reduction in WGA binding, suggesting that axons targeted to both ordinary and sexually dimorphic glomeruli carry a WGA-binding epitope that is not N-linked to protein. In short, axons targeted to the ordinary glomeruli in males differ from MGC axons and also from LFG + ordinary female axons in the nature of their WGA-binding moieties; only male axons targeting ordinary glomeruli display N-linked glycoproteins. The natures of the WGA-binding components of the other groups are being investigated; initial results suggest they may be glycolipids. NIH P01-NS28495.

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NCS-1 IN THE DEVELOPING OLFACTORY SYSTEM.

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The superfamily of EF-hand calcium-binding proteins include the expanding family of intracellular neuronal calcium sensor (NCS) proteins. The NCS family comprises 5 subfamilies; recoverins, frequinins; VILIPs, ce-NCS-2 and GCAPs, which are widely but differentially expressed in the nervous system and have been implicated in modulating signal transduction pathways. Members of the recoverin, VILIP and GCAP subfamilies are expressed in the rat olfactory system. Here we report the presence of NCS-1, the mammalian homolog of frequenin, in the developing mouse OE and OB. NCS-1 is highly enriched in OSN dendritic knobs as well as in developing OSN axons en route to the OB. In the OB, NCS-1 is found in OSN axons as well as in dendrites in the glomerular, external plexiform and granule cell layers. Expression in OSN axons is highest early in olfactory pathway development, prior to glomerular formation although expression persists in OSN axons into the postnatal period. NCS-1 has been previously implicated in modulation of calcium/calmodulin dependent enzymes involved in neuronal signal transduction and may substitute and/or fine-tune calmodulin signaling function. Intriguingly, various NCS proteins (including NCS-1) have been shown to modulate G-protein-coupled receptor kinases in vitro. As odorant receptors are G-protein coupled, the presence of NCS-1 in the dendritic knob suggests a role for this molecule in the regulation of olfactory transduction. Moreover, NCS-1 expression in the axons of OSNs is provocative in light of the suggestions that: 1) OR proteins are present in OSN axons; and 2) are necessary for appropriate targeting. Thus, NCS-1 may modulate odorant receptor mediated axon guidance. Supported in part by DC00210 and DC03887 to CAG

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CADHERINS AND CATENINS IN THE DEVELOPING OLFACTORY SYSTEM.

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During olfactory development, OSNs in the olfactory epithelium extend axons to the olfactory bulb (OB), which target glomeruli and form synapses with remarkable specificity. Recent studies have indicated that odorant receptor proteins have a role in this process but are insufficient for correct targeting. Thus, other guidance molecules are likely to be acting to help establish the primary olfactory projection. Good candidate molecules for guiding the formation of specific synapses in the developing OB are the members of the cadherin family. Cadherins are a large family of homophilic adhesion molecules that, together with their intracellular binding partners the catenins, are involved in many aspects of neural development, most notably specific target recognition and the formation of proper circuits. To investigate whether members of these families are involved in primary olfactory pathway development, we undertook a PCR based screen of cadherin expression. To date, we have identified 8 cadherins expressed in the developing mouse olfactory bulb. Immunolocalization studies of two of these cadherins, CDH1 and CDH2, demonstrated distinct patterns of expression in the developing olfactory nerve and glomerular layers of the OB. Moreover, catenins, which are critical binding partners of the classic cadherins, are also expressed in distinct patterns in the olfactory bulb. Together, these data provide good evidence for cadherins' involvement in the establishment of the primary olfactory projection. Supported in part by DC00210 and DC03887 to CAG.

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IN VITRO EVIDENCE THAT DIFFUSIBLE FACTOR(S) RELEASED BY OLFACTORY EPITHELIUM PROMOTE DENDRITE EXTENSION OF MITRAL CELLS

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Multiple lines of evidence indicate the olfactory nerve is important for the differentiation and the survival of the mitral cells during development. However, the regulatory mechanism for this process is not entirely clear. In this study, we directly tested the role of the mouse olfactory nerve on the differentiation of the mitral cells using an in vitro system. Mitral cells are born between E11-E13 in mice. MAP2 immunostaining and DiI tracing experiments suggest that differentiating mitral cells initiate their primary dendrites around E14, although their axons have reached piriform cortex by this age. We have taken advantage of the targeted mutation mice which express green fluorescent protein in early differentiating mitral cells (kindly provided by A. Walz and P. Mombaert) to evaluate mitral cell differentiation in vitro. When olfactory bulb(OB) explants from E11 to E13 are cultured over the olfactory epithelium(OE) for 5 days, the mitral cells are greater in number than that of the OB explant cultured alone. Moreover, mitral cell neurites are longer in OB+OE cultures than that of the OB alone. This data suggests that the presence of the olfactory epithelium supports the survival and the neurite extension of the early differentiating mitral cells. We did not observe penetrating olfactory axons into the OB explants. Therefore, it is likely that diffusible factors are involved in this process. Further studies are in progress to examine the molecular nature of the neurite promoting factors. Supported by NIH grant R03 DC04771-01A1

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ROLLER TUBE COCULTURE OF OLFACTORY EPITHELIUM AND BULB SUPPORTS CHRONIC NEURON SURVIVAL

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The first synaptic exchange in the olfactory pathway presents the odorant experiences of olfactory receptor neurons in the nasal cavity to the central neurons of the olfactory bulb. This seminal exchange takes place in a structurally complex environment called the olfactory glomerulus. We have undertaken to better understand the glomerulus and the first olfactory synaptic exchange by creating a culture system composed of olfactory epithelium and bulb. Tissue is collected from either 1-2 day old mice or late gestation dog fetuses. Extreme caudal epithelium (presumptive olfactory epithelium) is dissected from underlying cartilage, including cribriform plate, severing olfactory nerve fibers. Olfactory bulb tissue cleaned of pia is placed adjacent to the epithelium on a small piece of aclar. The explants are held in place by a plasma clot. The coculture is placed in a tube with medium and incubated while continuously rotating in a drum roller. To date we have learned that this method supports the survival of peanut agglutinin+/MAP2+ /beta-tubulin+ olfactory receptor neurons for more than six weeks. PNA- /MAP2+ /tubulin+ neurons also survive and elaborate extensive fine processes, some with axonal morphology. GFAP+ astrocytes are also present. These preliminary results suggest that long term coculture of olfactory epithelium and bulb is a viable model for learning more about the initial olfactory synapses.

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DIFFERENTIAL EXPRESSION OF CAMKII IN THE MAIN AND ACCESSORY OLFACTORY BULBS

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Recent evidence indicates that the MOB and AOB have significant differences in both their cytoarchitectures and the mechanisms by which they process stimulus information. To date little is known about whether or how molecular events can differentially regulate their formation and function. CaMKII is highly enriched at synapses and is implicated in the development and function of the visual and somatosensory systems. CaMKII is also highly expressed in the olfactory system, but its function remains largely unknown. We have examined CaMKII expression patterns in both the MOB and AOB of mice by immunohistochemistry. The distinct laminar expression of CaMKII was similar in these two tissues. In both MOB and AOB, high levels of CaMKII immunoreactivity were detected in the EPL and the GCL. In contrast, CaMKII was absent in the nerve layer. In the GCL, CaMKII was highly expressed in the majority of GABAergic granule cells in both MOB and AOB. One major difference in CaMKII expression between MOB and AOB occurred in the GL. In the GL of the MOB, CaMKII expression levels were minimal, with CaMKII absent in the majority of periglomerular cells. In contrast, CaMKII in the AOB was highly expressed in the majority of GABAergic PG cells. Another difference occurred in the glutamatergic mitral/tufted cells. In the mitral/tufted cells in the MOB, CaMKII immunoreactivity was not detected. In contrast, CaMKII was expressed in some (if not all) mitral/tufted cells in the AOB. Taken together, our results suggest that CaMKII may differentially regulate the development and function of dendrodendritic microcircuits in the GCL in the MOB and AOB. Additionally CaMKII may also play a role in the plasticity and signal processing in the GL of the AOB.

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TELENCEPHALIN EXPRESSION IN THE DEVELOPING MOUSE OLFACTORY BULB

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Telencephalin (TLCN; ICAM-5) is a cell surface glycoprotein believed to be important in the early outgrowth and differentiation of dendritic processes. Recent studies show that it is targeted to the somatodendritic domain of hippocampal pyramidal cells in vitro and that it may function, in part, via homophilic binding between neurons and heterophilic binding between neurons and leukocyte beta(2)-integrins. To understand the role that TLCN may play in mouse olfactory bulb, we have used double-label immunohistochemistry with TLCN to establish its cellular and temporal expression. At postnatal day 4 (P4), and in the adult, TLCN appears localized to granule cells. It colocalizes with GAD-65 and the morphology of the stained cells is consistent with granule cells. Developmentally, TLCN was first detected in the olfactory bulb at embryonic day 15 (E15). Of interest, GAD-65 was not present at E15 suggesting that TLCN is among the earliest molecular markers of developing granule cells. Staining with MAP-2 and calretinin at additional ages showed the strong expression of TLCN throughout the granule cell somatodendritic axis. There was no evidence of TLCN staining in other olfactory bulb neurons. The results appear consistent with the hypothesis that homophilic binding mechanisms that include TLCN may influence early migration, differentiation and development of olfactory bulb granule cells. Supported in part by NIH DC00210, DC03887 and NS10174.

SUPEROXIDE DISMUTASE IMMUNOREACTIVITY IN THE HUMAN OLFACTORY BULB: EFFECT OF AGE IN ALZHEIMER'S DISEASE PATIENTS

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We previously demonstrated that immunoreactivity (ir) for nitrotyrosine (NT), a peroxynitrite-mediated protein modification, is more intense in the olfactory bulbs (OBs) of patients with Alzheimer's disease (AD) compared to non-demented elderly (NDE) controls. Our aim was to investigate ir for Cu/Zn and Mn superoxide dismutases (SODs), which reduce the level of superoxide radicals, in the OBs of NDE and AD subjects. Peroxidase immunostaining was performed on OBs obtained at autopsy from subjects with short post-mortem intervals (5 h or less) whose status was confirmed neuropathologically. All NDEs, who were in the oldest-old (85+ years, n=6) age group, showed robust ir for both SODs, with strong staining in glomeruli, periglomerular (PG) cells, and capillaries. Robust ir was also observed in AD patients in the young-old (65-74 years, n=2) and old-old (75-84 years, n=9) groups with the same distribution as in NDEs. In the oldest-old ADs (n=5), ir was very weak, with almost no glomerular staining and faint ir in PG cells. The oldest-old ADs also had the most intense NT ir, consistent with a reduction in the ability to reduce superoxide radical levels. We hypothesize that the OBs of the oldest AD patients may incur more oxidative damage in part because of failure to upregulate SODs in response to high superoxide radical levels and also because SOD activity may be reduced by increased NT modification. Support: NIH NIA 1 R01 AG16345 (MLG). The Alzheimer's Disease Research Center, which provides tissue and neuropathological analyses, is supported by NIH P50-AG05144.

APOPTOTIC CELL DEATH IN THE AGING OLFACTORY EPITHELIUM

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Apoptosis of olfactory receptor neurons (ORN) and their precursors occurs at a baseline rate even in the absence of obvious disease, likely secondary to their exposed location in the nose. This neuronal death is matched by the regeneration of ORNs from progenitor cells in order to maintain an adequate number of ORNs necessary for olfactory sensation. In humans, studies have demonstrated an age-related decline in ORN number, evidencing ultimate failure of these homeostatic mechanisms. The central question remains whether this age-related failure is the result of decrease in proliferation or an increase in apoptosis. The current study utilizes the TUNEL technique to quantitate the level of apoptosis in non-operated 12 week, 18 month and 33 month old F344xBN rats. To assess the effects of injury as a function of age, apoptosis is being quantified in the three age groups at 2 days and 9 days post axotomy. Preliminary results indicate that the level of apoptosis is age-dependent, and this may, in part, account for age-related olfactory dysfunction in humans. Supported by NIA grant R03 AG19965-01.

THE IMPACT OF AGING AND MEDICATIONS ON FUNCTIONAL CHARACTERISTICS OF HUMAN OLFACTORY NEURONS.

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Olfactory receptor neurons (ORNs) provide an unique opportunity to study the neurophysiology of human aging as they exhibit features common to CNS neurons, can be obtained via biopsy from live subjects and respond to odorants with changes in intracellular calcium that are linked to distinct transduction pathways. Age-associated changes in calcium regulatory proteins occur in the CNS, and altered calcium homeostasis could contribute to age-associated losses in olfactory ability. To study this possibility, we evaluated the functional characteristics of >1000 ORNs obtained via biopsy from 200 subjects (age: 18 - 84) whose medical history and olfactory function were also documented. While there were age-related declines in olfactory sensitivity, we found: 1) no age-related loss in the number or viability of ORNs; 2) an age-associated increase in ORN response frequency; 3) ORNs from older subjects were more likely to respond to multiple, unrelated odorants. These findings were not associated with usage of a particular type of medication or prior disease. Our results suggest that age-related losses in olfactory sensitivity might be due to increased 'noise' in the input to the olfactory bulb, or to chronic adaptation and reduced ORN activation. These ORNs thus provide a valuable tool to study the effects of aging, medications and disease on neuronal calcium homeostasis and signal transduction, and to identify targets for therapeutic agents. Funded in part by NIH 00214 and NIH 00566.

MHC-DERIVED ODOR PREFERENCES IN AGING FEMALE RATS

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The purpose of this study was to determine if old female rats manifest MHC-based odor preferences even when they are no longer fertile. MHC-based odor preferences were assessed both in the context of social odor preference and mate choice. By focusing on females, the role of ovarian status on MHC-based preferences could be assessed. 10 PVG.RT1u and 10 PVG.R8 rats were used. These strains are genetically identical except for a difference at one MHC locus. Partner preference paradigms were used to determine both social preference and mate choice. Social preference was measured by the amount of time female's spent with syngenic (MHC-same) and congenic (MHC-dissimilar) males. Mate choice was based on sexual behaviors engaged in by the female and male. Only the PVG.R8 females displayed a social preference for the MHC congenic males ($p < 0.01$), but this preference was unaffected by ovarian cyclicity. All females had reached reproductive senescence by the time mating tests were conducted. In spite of this, they produced significantly more mating behaviors in the presence of the MHC congenic males than the syngenic males ($p < 0.05$). Our findings (1) demonstrate that MHC-derived odors enable social preferences and mate choice decisions of rats, with a bias towards dissimilar MHC individuals and (2) suggest that there is variability in expression of this selection mechanism. MHC-based preferences, nevertheless, may be part of an enduring, adaptive system with multiple functions throughout the life span. More research is needed to elucidate the role of the MHC in female social preference and choice behaviors. This research was funded by an NIMH MERIT Award to MKM.

ODOR MEMORY IN AGING RATS

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To study odor memory abilities in rats of different ages, 6 months old (n=5), 19 months old (n=5) and 26 months old male rats (n=8) were trained in an olfactometer to discriminate two odors. After successful training they were trained on two subsequent days to discriminate 4 odors (two S+, two S-). Performance of the 6 months old rats was significantly better than that of the older animals; the young rats reached 91% correct responses (cr), the older animals 83% cr and 78.75% cr, respectively in the first 20 trials of the session. However, at the end of the second day discrimination performance of the aged rats surpassed the 90% cr level. The following two days rats were trained on a second discrimination problem. Following three days of intermission, animals were retrained on the first problem for two days, afterwards a third combination of 4 odors was offered. After another break of 11 days, a memory test was conducted for the first discrimination problem. In this memory test, rats were not rewarded for responding correctly to presentations of S+. All age-groups reached the 90% cr. No significant difference between the ages could be detected. Six months later, animals were reaccustomed to the experimental procedure. Animals were now 12 months (n=4) and 26 months (n=4) old. Performance in the memory test of the younger rats was 83.7% on the average, the older ones reached 75% (no significant difference). We conclude that in a four-odor-discrimination task older rats need more repetitions to learn the meaning of odors as reinforced or not-reinforced. Yet, in comparison to young rats, long-term odor memory is not diminished.

DECREASED ACTIVATION IN PRIMARY OLFACTORY AREAS OF OLDER SUBJECTS IN RESPONSE TO RETRONASAL OLFACTORY STIMULATION: A REGION OF INTEREST ANALYSIS OF FMRI DATA

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Inter-individual differences in brain anatomy and function often limit detection of activation in functional Magnetic Resonance Imaging (fMRI) data processed with group analysis. Visual identification of anatomical regions is subject to experimenter's interpretation. The present study used a reproducible Region Of Interest (ROI) approach to identify specific areas of differential activation between young and old subjects in response to retronasal olfactory stimulation with odorants dissolved in water. Twelve subjects, 6 young (3M, 3F, mean age: 26.5 yrs.) and 6 old (3M, 3F, mean age: 78.5 yrs.) underwent an fMRI session with a 1.5 T scanner and did 6 functional runs, each with one odorant alternating with water, 18s ON, 75s OFF. Functional runs were processed by correlation with a perception-based template with AFNI (Cox 1996). ROIs were extracted from Talairach database implemented in AFNI software and included piriform cortex, amygdala, entorhinal cortex, hippocampus, parahippocampal gyrus, insula, orbitofrontal cortex (OBF) brodmann area (BA) 11, OBF BA 47, anterior and posterior cingulate gyrus. Signal was averaged in each ROI for each subject and each stimulus. Repeated measures ANOVA showed that old subjects had significantly less activation in primary olfactory areas – piriform cortex, amygdala and entorhinal cortex ($F=7.23$, $p=0.014$), in agreement with olfactory deficits observed in old subjects with psychophysical measures and event related potentials. Funding: NIH grant AG04085 to C.M.

ODOR DETECTION THRESHOLDS IN YOUNG AND OLD MICE.

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Olfactory deficits are common in human aging, although the neurobiological mechanisms responsible are not well understood. In order to study this problem, we are testing olfactory function in young and old mice. Young (4 months) and old (24 months) C57BL/6JNia mice were water deprived and trained in an automated olfactometer to discriminate 0.01% ethyl acetate vapor (EtAc) from clean air using a simultaneous-cue, forced choice procedure. A correct response constituted a nosepoke in the port containing the odorant stimulus and was rewarded with a drop of water. Well-trained animals were tested for discrimination of different concentrations of EtAc versus air using a staircase procedure. There were no significant differences in correct scores between young and old mice at any odorant concentration. Scores for both groups were significantly above chance at 0.001% and 0.0003% EtAc and not above chance at 0.0001% and 0.00003%. This implies a threshold at between 0.0003% and 0.0001% for both young and old mice. There were significant differences between the young and old mice in response latencies. Both groups responded equally rapidly on discriminations between low EtAc concentrations and clean air, i.e., discriminations that could not be made accurately. However, old animals took longer to respond on trials in which the two stimuli were distinguishable. The equivalence of latencies in young and old mice at the lowest concentration would suggest that motor/motivational factors are not responsible for the young-old difference at high concentrations. Rather, sensory or cognitive factors seem to separate the young and old animals; nevertheless, accuracy was equivalent in the two groups. (Supported by NIA and AFAR).

ADDITIONAL INSIGHTS INTO THE INFLUENCE OF AGING ON ODORANT QUALITY PERCEPTION

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Investigations of the effects of aging on odorant perception have revealed changes in facets such as detection threshold, hedonic tone, and trigeminal perception. However, little attention has been given to the effect of age upon odorant quality perception. To investigate the effects of aging on odorant quality perception, young (n=17) and elderly (n=14) subjects rated the perceptual dissimilarity of 45 odorant pairs using the Labeled Dissimilarity Scale (LDS) (Kurtz et al., 2000). All subjects scored at least 32/40 correct on the UPSIT. A people space analysis indicated that the young and elderly grouped in different locations in the space, suggesting a shift in odorant quality perception. This shift was not due to differences in mean perceptual intensity nor to differences in scale usage. On the other hand, it appears to be due to shifts in the perception of specific pairs of odorants. In each of these pairs, odorant dissimilarity was smaller in the elderly than in the young. Ratings of dissimilarity for the other pairs did not differ between the young and the elderly. From these data, we conclude that the aging process influences the quality perception of some, but not all odorants.

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RELATIONSHIPS BETWEEN FOOD PREFERENCE AND INTAKE ACROSS THE LIFESPAN: NEW FINDINGS FROM NHANES III AND USDA SURVEYS

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Recent data from our lab (Snyder et al., 2001) and NHANES III (Kant, 2000) indicate that preferences for high-fat and bitter foods increase with age, while those for sweets decline with age for women but remain stable for men. While diet is known to affect disease risk, the extent to which food preferences predict diet is unclear. To assess this, we compared our preference data for 26 foods (N = 2330) with 24-hour food diaries from NHANES III and the USDA Continuing Survey of Food Intakes, as well as NHANES III food frequency data. Intake measures for each food were averaged by age (decade) and food group (sweet, salty, bitter, fat). Bitter foods showed the most stable agreement between preference and intake, which increased with age; beer intake, however, declined over time, perhaps due to concerns about health risk. For similar reasons, fat intake did not show the clear age-related increase associated with fat preference; also, while salt preference was relatively stable over time, consumption of some salty snacks declined with age. Generally, sweet foods were preferred and consumed less with age, but ingredient sweets (e.g., sugar, honey) were eaten more, perhaps as sensory compensation for diets containing less fat and/or salt. As a whole, food preferences loosely predict intake, and agreement is best when foods are analyzed individually. Because individual foods in the same nutritional group differ broadly in terms of sensation, hedonics, and perceived healthfulness, we conclude that nutritionally-based food groups may not be a practical means of studying food preference and intake. (DC 00283)

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AGE-RELATED DIFFERENCES IN FAT PERCEPTION AND DIETARY BEHAVIORS

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Age-related differences in taste, fat perception and dietary fat behaviors were examined in 41 younger (24±4 y) and 42 older (67±7 y) women, the latter were post-menopausal and not on estrogen replacement. Subjects used the general Labeled Magnitude Scale to rate: creaminess of sampled high-fat foods (cheddar cheese, icing, cream cheese, mayonnaise, milk chocolate), intensity of tastants (1 M NaCl, 1 M sucrose, 32 mM citric acid, 1 mM QHCl) perceived by whole mouth and painted on areas innervated by cranial nerves (CN) VII and IX, and degree of acceptance of 22 high-fat foods, which formed a statistically reliable group. A frequency questionnaire assessed intake of 40 high-fat foods. Data were analyzed with t-tests (significance criteria $p < 0.05$). Younger and older women were matched for taste genetics (6-n-propylthiouracil bitterness, fungiform papilla density). Older women showed reduced CN VII taste for salt, sweet and bitter; however, they reported greater whole mouth saltiness from NaCl and creaminess from sampled high-fat foods. Older women reported greater preference for the fat group and greater intake of some high-fat foods (condiments, meats). Younger women consumed high-fat "fast-foods" most frequently. These findings support age-related vulnerability for CN VII taste and heightened whole mouth creamy and concentrated salty sensations, possibly from taste-trigeminal interactions. In aging, oral sensory changes may explain heightened fat preferences and interact with socio-cultural factors to influence fat intake. (NIA RO3AG18619 and Donaghue Foundation DF 98-056 funded)

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FLAVOUR-TEXTURE INTERACTION IN MODEL CHEESE WAFFLES

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Elderly (>60 yrs) and young (18-35 yrs) consumers evaluated the intensity of several flavour and texture attributes and the pleasantness of eight model cheese waffles differing in texture and flavour to study the flavour-texture interaction effects. The waffles were prepared according a full factorial design with two levels of cheese flavour, of gluten, and of fat. Anova showed that increasing the cheese flavour level increased the perceived fattiness, saltiness and cheesiness in the waffles. It decreased the perception of the attributes dry, elastic, swallowing effort and remains after swallowing. The pleasantness increased with the increased flavour level for the elderly only. Increasing the fat level of the waffles increased the perceived intensity of fatty, dry, swallowing effort, salty, cheesy and remains after swallowing, and it decreased the pleasantness and the pleasantness. Lowering the gluten level decreased the perception of fatty and airy and increased the perception of dry, elastic, swallowing effort and remains after swallowing. It had no effect on the flavour attributes, but it decreased the pleasantness of the waffles. A flavour-fat interaction and a flavour-gluten interaction was found for the attribute cheesy. No differences in these interactions were found between the elderly and the young. Age effects were found on flavour and texture perception, as well as on the perceived pleasantness. The elderly assessed most of the flavour and texture attributes as less intense than the young. The elderly showed clear differences in preferences whereas the young did not. Financial support from the EC Quality of Life Framework Programme QLK-1999-00010

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PARKINSON'S DISEASE AS POSSIBLE CAUSE OF IDIOPATHIC OLFACTORY LOSS

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Olfactory loss is an early sign of Parkinson's disease (PD). It is unclear whether and how far olfactory dysfunction precedes first clinical signs related to the motoric system. The present study aimed to investigate whether "idiopathic" olfactory loss might relate to the presence of Parkinson's disease. A total of 18 patients diagnosed with "idiopathic" olfactory dysfunction participated in this investigation (12 men, 6 women; mean age 59 years, age range 35-80 years). Using transcranial sonography 6 patients (33%) exhibited signs of nigral degeneration. In addition, these patients had subtle clinical signs of motor dysfunction. These data indicate that, in many patients, "idiopathic olfactory loss" may be an early sign of PD. Consequently, in clinical practice cases with "idiopathic olfactory" should be seen by an experienced neurologist. Further, while more research is needed, the combined investigation of olfactory function and nigral degeneration might become a valuable tool in the early diagnosis of Parkinson's disease.

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OLFACTORY DYSFUNCTION IN DEGENERATIVE ATAXIAS

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Recent studies suggest the possibility that the cerebellum may play a role in higher-order olfactory processing. In this study, we administered the University of Pennsylvania Smell Identification Test (UPSIT) to patients with ataxias due primarily to cerebellar pathology (spinocerebellar ataxias and related disorders), and to patients with Friedreich's ataxia, an ataxia associated with loss of afferent cerebellar pathways. UPSIT scores were slightly lower in both patient groups than in the control subjects, but no differences were noted between the scores of the Friedreich's and the other ataxia patients. Within the Friedreich's ataxia group, the smell test scores did not correlate with the number of pathologic GAA repeats (a marker of genetic severity), disease duration, or categorical ambulatory ability. UPSIT scores did not correlate with disease duration and correlated only moderately with ambulatory status in the patients with cerebellar pathology. This study suggests that olfactory dysfunction is a subtle clinical component of degenerative ataxias, in accord with the hypothesis that the cerebellum or its afferents plays some role in central olfactory processing. Supported, in part, by NIH Grants PO1 DC 00161, RO1 DC 04278, RO1 DC 02974, and RO1 AG 27496 and a Beeson Scholar Award from AFAR (DRL).

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CORRELATES OF ODOR DISCRIMINATION, IDENTIFICATION AND RECOGNITION MEMORY TASK PERFORMANCE IN PATIENTS WITH EPILEPSY

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Deficits in odor discrimination, identification and recognition memory ability exist in pre- and post-surgical patients diagnosed with epilepsy. Whereas patients with right mesial temporal lobe and orbitofrontal cortex focus and resection are consistently found to present with olfactory deficits, the literature inconsistently reports deficits in left temporal lobe patients. The present study assessed the relationship between age at onset of illness, duration of illness, frequency of seizures, symptoms of depression, seizure focus, unihornal assessment, attention and naming ability with odor discrimination, identification and recognition memory ability in patients with epilepsy. Patients diagnosed with complex partial seizures of left and right temporal lobe origin and seizures of generalized or multifocal origin all confirmed with extended inpatient video/EEG monitoring techniques were assessed. A modified version of the UPSIT was adapted to also assess unihornal odor discrimination and short-delay recognition memory ability (20-minute delay). Preliminary results indicated that poor attention and naming ability were significantly associated with reduced odor discrimination and identification test performance for all groups. Interestingly, younger age at onset of seizures was related to lower odor identification performance. Further, elevated symptoms of depression were correlated with increased number of false positives on the recognition memory task. Seizure and non-olfactory cognitive variables may explain some variability found in previous studies.

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SIGNIFICANT HYPOSMIA IN CYSTIC FIBROSIS

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Little is known about the olfactory functions in Cystic Fibrosis (CF), the most common lethal autosomal recessive disease that affects Caucasians. The clinical manifestations of the disease include pancreatic enzyme deficiency with malabsorption and chronic progressive obstructive pulmonary disease while the most frequent otolaryngologic manifestations are chronic sinusitis and nasal polyposis which affects the sense of smell. Preliminary results show (in a group of 117 patients 5 to 62 years of age examined year 2000 & 01) that hyposmia is frequent in this population. Olfactory sensitivity test (i.e. absolute threshold for Butanol) was pathological in 64% while the identification test showed hyposmia in 39%. The olfactory functions were further reduced by the presence of nasal polyps. The frequency of nasal polyposis was estimated to 36% with an endoscopic technique. Surprisingly, only 14% of the patients did experience any kind of lowered smell sensitivity on a continuous basis. 73% of the patients declared, at the time of the examination, that their sense of smell was normal. The new identification test used for the children aged 5-14 years (The Swedish Smell Identification Test for Children (SSIT-C)) is presented by Anna Hallberg at the present meeting.

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CHEMOSENSORY PERCEPTION IN ELDERLY LUNG CANCER PATIENTS ON CHEMOTHERAPY

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The purpose of this study was to assess the effect of chemotherapy on taste and smell perception in older lung cancer patients, and to examine the relationship of the patient's chemosensory perception to nutrition, quality of life, and immune measures. Thirty-three lung cancer patients aged 68 years (SD 6.72) were evaluated one month after commencing chemotherapy. Taste detection and recognition thresholds (DT and RT) were obtained for NaCl and sucrose, and odor thresholds for phenethyl alcohol and menthol. Self-reported taste and smell disturbances were frequent in this group with 42% reporting a bad taste in their mouth, and 36% reporting a change in the intensity of odors. Sixty-seven percent also reported dry mouth or a change in their saliva since beginning chemotherapy. The measured recognition thresholds for both tastants and odors were significantly elevated. The RTs for NaCl and sucrose were 0.087 M (SD=0.153) and 0.12 M (SD=0.136), respectively with two subjects having no RT for NaCl, and two subjects having no RT for sucrose. Evidence of dysgeusia was found with mislabeled tastes at suprathreshold concentrations. There was a correlation between the RT values for NaCl and loss of appetite (Pearson correlation = 0.35, $p < 0.05$) and weight loss (Pearson correlation = 0.47, $p < 0.01$). There was a correlation between a person's self-reported odor sensitivity and loss of appetite (Pearson correlation = 0.41, $p < 0.05$), weight loss (Pearson correlation = 0.38, $p < 0.05$) and their overall nutritional assessment score (Pearson correlation = 0.47, $p < 0.01$).

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SMELL LOSS IN NASAL-SINUS DISEASE

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Chronic diseases of the nose and/or paranasal sinuses (NSD) underlie approximately 30% of the cases of smell loss seen in patients presenting to specialized centers for chemosensory evaluation; however, the actual prevalence of smell loss in those who suffer from NSD is not known. Moreover, although it now seems clear that simple mechanical obstruction does not explain this form of loss, the critical pathologic mechanisms and specific characteristics of NSD that lead to smell loss have yet to be identified. To begin to address these questions, we are conducting a large-scale study of new patients presenting with nasal/sinus complaints to a general practice in otorhinolaryngology, both before and after therapeutic intervention. Olfactory function is assessed via measures of threshold sensitivity to phenylethyl alcohol obtained separately for each nostril. Findings to date suggest that although a substantial proportion of patients suffering from chronic nasal-sinus disease evidence at least unilateral olfactory difficulty (25/65: 38.5%), only 10.8% (7/65) show significant bilateral loss. Because of the very high prevalence of nasal-sinus disease (125.5 per 1000, National Center for Health Statistics, 1996), this could still represent a large number of people with olfactory problems (>4 million). Nonetheless, our findings suggest there are very specific components of this diverse disease process that impact olfaction. Supported by NIH P50 DC00214.

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OLFACTORY AND NEUROPSYCHOLOGICAL DEFICITS IN PATIENTS WITH ALCOHOL DEPENDENCE

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Previous studies indicate that even nonamnesic and nondemented alcoholic patients show olfactory deficits. These deficits have been shown to be only partially reversible with abstinence. A few findings indicate that cortical structures play an important role in this sensory loss. The aim of the present study was to assess the association between olfactory dysfunctions and neuropsychological deficits in alcohol dependent patients (DSM-IV), who do not meet criteria for alcoholic dementia or amnesic syndrome. Olfactory (Sniffin' Sticks) and neuropsychological domains (general mental ability, executive functions, memory and verbal fluency) were evaluated in alcoholic patients and healthy controls, matched for age, sex and smoking status.

Results indicate that alcohol dependent patients as a group were impaired, relative to controls, on all olfactory domains and on many of the neuropsychological tests. Whereas group differences in identification remained significant after controlling for olfactory acuity, controlling for quality discrimination on identification resulted in the effect of group differences disappearing, indicating the crucial role of quality discrimination for group differences in olfactory identification. Although some of the neuropsychological tests showed a correlation with olfactory deficits, results suggest that olfactory deficits in alcohol dependent patients can not be attributed to these cognitive dysfunctions, but might be due to a sensory loss relatively independent of disturbances in some neuropsychological domains.

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AN UNBLINDED TRIAL OF ALPHA-LIPOIC ACID IN THE TREATMENT OF OLFACTORY LOSS FOLLOWING INFECTIONS OF THE UPPER RESPIRATORY TRACT

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The study aimed to investigate the potential therapeutic effects of alpha-lipoic acid (ALA) in olfactory loss following infections of the upper respiratory tract (URTI). Possible mechanisms of actions include the release of nerve growth factor or anti-oxidative effects. A total of 23 patients participated (age range 22-79 years; mean duration of olfactory loss: range 4-33 months); 19 of them were hyposmic, 4 were anosmic. ALA was prescribed for an average of 4.5 months at a dose of 600 mg/day. Olfactory function was assessed using the "Sniffin' Sticks" (phenyl ethyl alcohol odor threshold, odor discrimination, odor identification). In addition to an extensive ENT examination, a detailed, standardized history was taken with special reference to the sense of smell. Following ALA treatment there was an improvement of olfactory function (MANOVA, p=0.013). Two of the anosmic patients became hyposmic. In 13 of the 19 hyposmic patients improvement of olfactory function was seen. Olfactory test scores decreased in 5 the 19 hyposmic patients. Interestingly, recovery of olfactory function appeared to be more pronounced in younger patients than in older patients (>60 years; t=2.56, p=0.018). At the end of treatment parosmias were less frequent (22%) than at the beginning of therapy. These results indicate that ALA may be helpful in patients with post-URTI olfactory loss. However, to judge the true potential of this treatment, the outcome of double-blind, placebo-controlled studies in large groups of patients needs to be awaited, especially when considering the relatively high rate of spontaneous recovery in post-URTI olfactory loss.

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ENVIRONMENTAL ODOR SENSITIVITY IN FEMALES: CAN IT BE INDUCED EXPERIMENTALLY BY REPEATED EXPOSURES TO LOW-LEVEL CHEMICALS?

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Environmental Intolerances/Multiple Chemical Sensitivities (EI/MCS) is more prevalent among women than men and is often triggered by detection of an odor. We investigated the hypothesis that objective gender differences in olfactory sensitivity following repetitive odorant exposures may underlie the increased prevalence of EI/MCS in females. In prior studies, we observed dramatic increases in olfactory sensitivity across repeated odor-threshold assessments among females, but not males. We hypothesized that such changes are accompanied by increased odor awareness, and can lead to increased frequency of health-symptom reports following exposures. 16 subjects (n=8/gender) received 11 controlled exposures to low-level concentrations of isopropyl alcohol (IPA) in an effort to induce increased olfactory sensitivity to the chemical and accompanying reports of transient symptoms consistent with EI/MCS. On Days 1, 6, and 11 of the sensitization period, subjects received ambient exposure to IPA to elicit mild transient symptom reports. Subjective reports of odor and health symptom intensity, and objective endpoints of skin conductance, respiration frequency, and expired CO₂ levels were collected throughout exposure. Consistent with predictions, many females exhibited small changes in both subjective stress and health symptom reports whereas males did not. Although odor perception and awareness appears related to EI symptomatology, the precise nature of the relationship has yet to be fully elucidated. Supported by ESRI and NIH RO1 DC 03704

THE MOLECULAR GENETICS OF HUMAN CONGENITAL GENERAL ANOSMIA

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While the genetics of blindness and deafness has been thoroughly investigated, much less is known about smell blindness - Congenital General Anosmia (CGA), except for the X-linked Kallmann Syndrome form. Through newspaper advertisement and a preliminary questionnaire that eliminated acquired anosmia cases, we obtained 162 presumed CGA subjects (~1/10,000 incidence). Of these, 62 were familial, in 24 families, a prevalence broadly consistent with autosomal recessive transmission. The largest family had 6 anosmics in 3 generations. A second family, with 3 CGA siblings, suggested putative non-Kallmann X-linked inheritance. A candidate gene table was constructed from genome data, based on animal models and on the notion that CGA is caused by genes affecting overall olfactory function. These include the cyclic nucleotide-gated channel alpha chain (*CNGA2*, Chr X), the olfactory G protein (*GNAL*, Chr 18), adenylyl cyclase III (*ADYCY3*, Chr 2), and the transcription factor *Olf1* (*EBF*, Chr 5). Homozygosity mapping for all CGA patients and segregating haplotype analysis in families are used as tools for causative gene identification. Short Tandem Repeat and high throughput mass-spectrometry-based Single Nucleotide Polymorphisms (SNP) analyses are employed for genetic linkage and association studies, to help decipher the molecular underpinnings of this chemosensory dysfunction. Supported by NIH (DC00305), the Crown Human Genome Center and the Israeli Ministry of Science.

THE CRANIAL I QUICK SNIFF™: A NEW SCREENING TEST FOR OLFACTORY FUNCTION

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The Cranial I Quick Sniff is a standardized, single-use, disposable odor presentation for the rapid assessment of olfactory function. Odor intensity and identification scores are obtained in a single trial. Designed for use by physicians, it lends itself to other smell screening applications. An individual administration (one page of a booklet) consists of an adhesive-backed data form and detachable scent sample. The scent sample is a liquid odorant sealed between two layers of plastic. It is handed to the patient who peels off the top plastic layer to expose the odorant. The patient rates smell intensity on a 0 to 10 scale ("none" to "strong") and identifies the odor from among 4 descriptors. Results recorded on the data form can be placed directly on the patient's chart. To establish normative data, the Quick Sniff licorice-scented variant was administered to 271 male and female subjective normosmics, ranging in age from 18 to 69 years. Mean odor intensity rating (\pm SD) was 6.7 ± 1.9 ; 11.4% of the sample misidentified the odor. Results will be analyzed by age and gender. Test-retest reliability was assessed with a separate sample of 42 undergraduates (8 men, 34 women). Mean age was 21.4 ± 3.2 years (range 18 to 36). The interval between tests was 68 days. Odor intensity scores at the two test dates were significantly correlated: Pearson $r = 0.51$, $p = 0.001$, $n = 42$. When 11 subjects who reported head cold or other nasal impairment at either test date are excluded from analysis, the test-retest correlation improves: $r = 0.57$, $p = .001$, $n = 31$.

COMPARATIVE STUDY BETWEEN THE T&T OLFACTOMETER AND THE ODOR STICK IDENTIFICATION TEST FOR THE PATIENTS WITH OLFACTORY DISTURBANCE

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A comparison between the thresholds from the Japanese standardized olfactory test (T&T olfactometer) and the identification rates from the Odor Stick Identification Test was made for 110 Japanese patients who have olfactory impairment ranging in age from 17 to 78 years. Both the detection and the recognition threshold for five odors were obtained using the T&T olfactometer. The identification rate for 13 odors familiar to Japanese was measured using Odor Stick Identification Test, which uses encapsulated odorants. An inverse correlation was observed between recognition threshold (T&T olfactometer) and identification rate (Japanese Stick test). Classification of patients into 5 levels of olfactory function along the spectrum from anosmia to normosmia is routinely performed in Japan using the recognition threshold of the T&T olfactometer. Although there was a good correlation for both test methods the Japanese Stick test did not discriminate between some categories of disturbance (e.g. normal and slightly impaired). We conclude that the Japanese Stick test is more useful as a screening test than for a more careful examination in a hospital setting.

FUNGIFORM PAPILLA (FP) NUMBER ASSOCIATES WITH DIETARY FAT BEHAVIORS AND SERUM CHOLESTEROL IN MIDDLE-AGED ADULTS

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Previously, we reported associations between taste genetics and risk of developing cardiovascular disease (CVD) in young adults. Presently, we examined this association in middle-aged adults (127 males and 30 females, mean age= 46 ± 8 SD) via a CVD risk appraisal at a manufacturing company. The number of FP served as a measure of taste genetics and was determined in a 6mm area on the left tongue tip using blue food coloring and 3x magnification. Subjects reported the degree of acceptance for fat foods (mayonnaise, steak, whipped cream, whole milk, sour cream, fried chicken, sausage, butter, gravy, sweets) on the general Labeled Magnitude Scale; these foods formed a statistically reliable group that ranged in ratings from "strongly dislike" to "very strongly like." Subjects also reported how frequently they consumed high fat foods (fried foods, red meats, processed meats, added fat, whole milk, desserts); these foods also formed a reliable group with an average intake of 5 times a week (range 1-11). Total cholesterol, determined from capillary blood, averaged 204 ± 35 (range 126-298). FP number (mean 18 ± 8 , range 5-40) showed significant negative correlations with fat acceptance, fat intake and serum cholesterol. In multiple regression analyses, the FP number prediction of serum cholesterol was independent from other CVD risks (eg, age, adiposity). These data support generalizability of the taste genetics and CVD risk association to middle-aged adults. (NIH DC00283, School of Allied Health, Pratt & Whitney, and Pfizer funded)

ARE 6-N-PROPYLTHIOURACIL (PROP) NONTASTERS AT RISK FOR HIGH BLOOD PRESSURE?

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The present study examined associations between PROP tasting, dietary factors that influence risk of hypertension (salt use, alcohol intake, adiposity) and blood pressure in 151 men and 41 women (mean age=44±8 y SD) during a cardiovascular disease risk appraisal at a manufacturing company. Subjects rated bitterness of PROP-impregnated paper on the general Labeled Magnitude Scale: the distribution suggested sampling nontasters, medium tasters, and supertasters. Trained technicians measured resting blood pressure (average systolic 120.2±14.0, average diastolic 76.9±10.1mm Hg), waist circumference (36.0±4.9 in), and height and weight for body mass index calculation (BMI; average 27.5±4.5 kg/m²). Employees reported use of added salt and alcohol consumption. In multiple regression analyses, PROP bitterness, age and BMI were significant independent predictors of systolic and diastolic pressure: salt use was not. In chi square analyses, those with elevated systolic blood pressure (≥130 mm Hg) were significantly more likely to taste PROP as ≤“moderately” bitter (ie, PROP nontasters) than those with normal blood pressure. Those who tasted PROP as least bitter were also significantly more likely to have greater waist circumferences and higher intakes of alcoholic beverages but less likely to report adding salt to foods. PROP nontasters may have greater risk of elevated blood pressure because of higher intakes of alcohol and dietary behaviors that increase overall and central body adiposity. (NIH DC00283, School of Allied Health, and Pratt & Whitney funded)

IDENTIFICATION OF A HUMAN BITTER RECEPTOR

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Taste is an important chemosensory modality that influences the choice and quantity of consumed food. Therefore, an understanding of the molecular mechanisms underlying taste sensation is important for strategies aiming towards an improvement of nutritional habits and the prevention of nutrition related diseases. Bitter taste not only protects humans from the ingestion of toxic compounds, but is also involved in the assessment of the palatability of certain food stuff and beverages, such as beer. The recent discovery of several members of a putative bitter receptor family, T2R, and the identification of one murine family member, mT2R5, as a receptor for cycloheximide (Adler et al., Cell 100, 693-702; Chandrasekar et al., Cell 100, 703-11; Matsunami et al., Nature 404, 601-4) provided the basis for a deeper insight in bitter perception. In an attempt to identify human bitter receptors, we searched the human genome database for sequences encoding T2R family members. So far, we found and cloned 24 human full length T2R sequences. Using a calcium imaging assay we identified a new cognate bitter tastant-receptor pair. The response was specific, dose-dependent, saturable and desensitized upon repeated stimulations. In human psychophysical experiments the same tastant displayed all the characteristics found in the in vitro studies. RT-PCR studies showed that the mRNA for the identified receptor is expressed in vallate papillae on the human tongue but absent from other tissues analyzed. Currently we are characterizing the ligand profile of the identified receptor.

MOLECULAR EVOLUTION OF THE MOSQUITO ANOPHELES GAMBIAECHEMORECEPTOR GENE SUPERFAMILY

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With completion of the *Anopheles gambiae* genome sequence it is possible to compare the repertoire of chemoreceptors it encodes with those encoded by the *Drosophila melanogaster* genome. These two flies represent the two major suborders within the order Diptera, last sharing a common ancestor 220-250Myr ago. *A. gambiae* has at least 44 odorant receptor genes in comparison to the 61 known for *D. melanogaster*. Phylogenetic analysis reveals that these gene families have undergone highly divergent evolution, presumably at least in part in adaptation to their divergent life histories. With the exception of the unusual DmOr83b and its highly conserved ortholog in *A. gambiae*, there are few simple orthologous relationships between single odorant receptor genes in the two species. Most convincing orthologous relationships involve multiple paralogs in each species. In addition, each fly lineage has evolved discrete unique subfamilies of genes that result from a combination of gene duplication and loss of the gene lineage in the other fly. Initial analyses of the gustatory receptors reveal similar evolutionary histories. This work is unfunded.

ODORANT RECEPTOR IDENTITY IMPARTS A SPECIFIC CODE TO AXONS OF OLFACTORY SENSORY NEURONS.

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Odorant receptors (ORs) transduce odorant-dependent signals within olfactory sensory neurons (OSN). These seven-transmembrane domain receptors are believed to be located on the cilia that project from the dendritic knob into the nasal cavity. ORs have been also suggested to be necessary for axonal convergence to specific target glomeruli. Gene-targeting technology in mice has shown that the OR possesses a code for this axon guidance mechanism. However, in the reported OR coding sequence replacements the donor receptor failed to impart the same identity to the newly marked sensory neurons: their axons projected to distinct glomeruli. We have continued using mouse gene-targeting in a large-scale replacement experiment with ORs from several zones and loci. We observe glomerular targeting that corresponds to the identity of the expressed OR sequence. We address how the zonal distribution of the OR affects the position by which glomerular formation occurs in the olfactory bulb. Two of the ORs that we studied are expressed in the same zone and differ in 11 amino acid residues, yet they target axons to distinct glomeruli. Using an in vivo structure-function paradigm we dissect the contribution of individual amino acid residues to axonal pathfinding, by comparing the glomerular targeting characteristics of 10 hybrids and by analyzing how they are affected by activity-dependent mechanisms. In addition we generated a set of mutant OR genes to understand the specific role of the OR protein in the axon guidance process. Taken together, our data show that the OR chosen by an OSN imparts its characteristic code to the navigating axons.

65 Slide Chemoreceptors: Genes and Proteins

DATA MINING, SEQUENCE ANALYSIS AND MOTIF DISCOVERY OF THE MOUSE OLFACTORY RECEPTORSZhang X.¹, Liu A.H.², Califano A.², Stolovitzky G.², Firestein S.¹¹*Biological Sciences, Columbia University, New York, NY;*²*Computational Biology Center, T. J. Watson IBM Research, Yorktown Heights, NY*

Olfactory receptors (ORs) encompass the largest gene superfamily in vertebrates. By a comprehensive data mining effort, we have identified the nearly complete mouse OR repertoire. Pooling this with the human repertoire, we now have over 2300 OR genes. Sequence analysis of such a large number of OR genes provides information at an unprecedented level. A high-performance, top-down, unsupervised system was applied to this large data set to exhaustively discover sequence motifs, which may be of structural or functional importance.

1) DATA MINING. We have identified 1296 mouse OR genes from the Celera mouse genome. OR genes are distributed in 27 clusters on all mouse chromosomes except 12 and Y. The mouse ORs can be divided into two classes, which can be further divided to 228 families phylogenetically. 2) SEQUENCE ANALYSIS (based on ~1300 intact, full length mammalian ORs). Hydrophobicity, volume, and variability plots identified conserved and variable regions and highlighted their chemical properties. Motif discovery was carried out at both the 1-D (adjacent amino acids) and 3-D (amino acids facing the same direction in the transmembrane regions) levels. Motifs with various support sets were discovered, such as patterns that were highly conserved in all ORs, class specific patterns, and patterns that only occurred in specific OR groups. The structural/functional significance of a few patterns is established, with the 3-D patterns in particular providing unique insights with regards to regions in the transmembrane domains.

66 Symposium Stem Cells and the Chemical Senses: Analytic and Therapeutic Approaches

INTRODUCTION: STEM CELLS AND THE CHEMICAL SENSES: ANALYTIC AND THERAPEUTIC APPROACHESSchwob J.E.¹ *¹Anatomy & Cellular Biology, Tufts University, Boston, MA*

The inherent capacity for neural repair and neuronal regeneration is limited in most of the nervous system, a major exception being the peripheral olfactory system. In order to obviate that unfortunate reality, a number of investigators are studying the capacity of stem cells, either tissue-specific or embryonic in origin, as substrates for facilitating recovery of structure and function throughout the CNS as well as in other tissues. Moreover, that the capacity for regeneration in the olfactory system is normally robust does not preclude the loss of olfactory function in some patient populations due to failures of the repair process. Thus, dysosmic/anosmic patients may benefit from the types of interventions that are being developed to mitigate CNS damage and stimulate repair. Finally, the behavior of olfactory stem cells may inform the analysis of other neural stem cells; indeed, olfactory stem cells may be an accessible source of neural stems that are broadly able to serve throughout the CNS. In order to highlight the interface between stem cells and the chemical senses, the symposium will emphasize 1) the initiatives that NIH is pursuing relative to stem cell research (Chiu); 2) the mechanisms regulating neural stem cell differentiation and proliferation (Rao); 3) the activation of endogenous stem cells in response to injury (Macklis); 4) the regulation of stem cell behavior in the mammalian olfactory system (Schwob).

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NIH POLICY AND FUNDING OF STEM CELL RESEARCHChiu A.Y.¹ *¹Repair and Plasticity Program, NINDS/NIH, Bethesda, MD*

The tremendous plasticity exhibited by stem and progenitor cells raises the possibility that they can be used to replace cells and restore vital functions that have been compromised by congenital disorders, developmental malfunction, injury or disease. In addition to cell and tissue therapy, the ability to selectively produce one or more differentiated cell types at will from stem cells would be of clinical importance in investigating the effects of drugs and environmental factors on human differentiation and cell function. Animal studies show that of the many types of progenitor cells competent to develop neuronal and glial features, embryonic stem (ES) cells, derived from embryos at the blastocyst stage, may have the broadest natural potential and a capacity for nearly unlimited self-renewal. On August 9th, 2001 President Bush announced that federal funds may be awarded for research using human ES cell lines that meet certain criteria. To implement this new policy, and to facilitate research using these cells, NIH created a Human Embryonic Stem Cell Registry (<http://escr.nih.gov>) that lists cell lines that meet the eligibility criteria. In addition, the NIH has great interest in supporting a wide variety of stem cell research ranging from their basic biology to pre-clinical applications in animal models of injury and disease. An understanding of factors that govern the activities of these cells is crucial in order to develop safe and effective treatments. Many of the initiatives developed by the NIH can be found at a stem cell website developed by the NINDS (<http://www.ninds.nih.gov/stemcells>). The hope is that federally-funded investigators will take full advantage of these new opportunities and explore the enormous promise of stem cells.

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STEM CELLS AND THE NERVOUS SYSTEMRao M.S.¹ *¹Stem Cell Biology Unit, NIA/NIH, Baltimore, MD*

Normal CNS development involves the sequential differentiation of multipotent stem cells. Alteration of stem cells number, self-renewal or proliferative capacity will have major effects on the appropriate development of the nervous system. In this talk I will discuss different mechanisms that regulate neural stem cell differentiation and proliferation. In addition I will describe the classes of precursor cells that have been identified and the factors that regulate the differentiation process. Similarities and differences with placodal cells will be discussed.

**69 Symposium Stem Cells and the Chemical Senses:
Analytic and Therapeutic Approaches**

INDUCTION OF NEUROGENESIS IN THE NEOCORTEX OF ADULT MICE

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Neurogenesis does not normally occur in postnatal cortex. Is this due to limits of endogenous precursors' potential, or lack of signals for neuronal differentiation? Prior results from our lab show that in regions of adult mouse cortex undergoing synchronous apoptosis of projection neurons, nearby cells upregulate genes that guide transplanted neuroblasts or precursors to undergo directed migration, differentiation, synaptic integration, and re-formation of long-distance projections. To direct the fate of endogenous precursors in adult cortex, we examined their differentiation when exposed to these signals in situ, without transplantation. Precursors can be induced to undergo progressive neuronal differentiation into mature neurons in a layer- and region-specific manner. BrdU+ newborn cells express Doublecortin, a marker of young neurons; Hu, an early neuronal marker; then NeuN, a mature neuronal marker, exclusively in regions undergoing targeted apoptosis of corticothalamic neurons (survive > 28 wks; $97 \pm 69/\text{mm}^3$ in exptl, 0 in ctrls). FluoroGold retrogradely labels new neurons forming long-distance corticothalamic connections. We can now also recruit other types of projection neurons. Together, our results demonstrate that precursors can be induced in situ to differentiate into cortical neurons and form appropriate long-distance connections in the adult brain. This suggests the possibility of neuronal replacement without transplantation of exogenous cells. Ongoing experiments aim at underlying molecular mechanisms; whether this neurogenesis can be increased; whether new neurons differentiate precisely; and whether newborn neurons join functional circuitry.

**70 Symposium Stem Cells and the Chemical Senses:
Analytic and Therapeutic Approaches**

THE ANALYSIS OF PROGENITOR AND STEM CELL CAPACITY IN THE MAMMALIAN OLFACTORY EPITHELIUM

Schwob J.E.¹ ¹*Anatomy & Cellular Biology, Tufts University School of Medicine, Boston, MA*

The peripheral olfactory system recovers after injury -- the olfactory epithelium (OE) reconstitutes to near-normal -- with a facility that is unique within the mammalian nervous system. Cell renewal in the OE is directed to replace neurons as they die in normal animals or at an accelerated pace following the retrograde degeneration caused by damage to the olfactory nerve or bulb. Neurogenesis persists because neuron-competent progenitor cells, including transit amplifying and immediate neuronal precursors, are maintained within the population of globose basal cells (GBCs). Notwithstanding events in the neuron-depleted OE, when both non-neuronal cells and neurons die following methyl bromide (MeBr) exposure, a multipotent progenitor is activated that gives rise to sustentacular cells and horizontal basal cells as well as neurons and GBCs. Immunohistochemical analyses and transplantation of FACS-sorted GBCs suggest strongly that the multipotent cell is a kind of GBC. Indeed, it seems that multipotent GBCs are mitotically active following bullectomy and generate neurons and nonneuronal cells after transplantation into MeBr-lesioned OE. From studies of epithelial explants and dissociated cells in culture, it appears that multiple growth factors, including TGF- α , FGF2, BMPs, and TGF- β s, are likely to regulate choice points in epitheliopoiesis, including progenitor cell fate. Transplantation is a powerful methodology for dissecting the contributions of growth factor signalling pathways and a potential means for therapeutic intervention in anosmic patients. Supported by NIH R01 DC02167.

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INTERACTION OF BURN BETWEEN CAPSAICIN, PIPERINE, AND ZINGERONE

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Presentation of one stimulus immediately before another may produce cross adaptation or sensitization of the response to the second stimulus. Such interactions may reflect common or interacting mechanisms in stimulus coding. Subjects were given continuous, 23-minute oral stimulation with one vanilloid irritant presented on filter papers, followed immediately by another for 23 minutes. Irritants were matched for maximum response. The following pairs were studied: Piperine \rightarrow Zingerone; Zingerone \rightarrow Capsaicin; and Zingerone \rightarrow Piperine. Piperine sensitized the response to Zingerone; by contrast, Zingerone cross adapted Capsaicin. After the transition from Zingerone to Piperine, the response decreased briefly, and then returned to its prior level. The time course of each response was predicted by the McBurney-Balaban model of adaptation, by which the response to a stimulus is represented as the sum of two processes: a phasic (or "change" detection) mechanism, and a tonic (or "level" detection) mechanism. The time constants of both processes were those determined in previous studies, but the gains for each mechanism differed between irritants. The interactions between irritants are those predicted as a linear sum of independent responses to the irritants. We conclude 1) that the psychophysical responses to these three irritants are mediated by tonic and phasic mechanisms with similar temporal properties and 2) that cross adaptation and cross sensitization may be explained as a temporal summation of independent irritant responses.

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DESENSITIZATION OF ORAL IRRITATION BY MUSTARD OIL AND RECIPROCAL CROSS-DESENSITIZATION WITH CAPSAICIN

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The magnitude of oral irritation elicited by pungent chemicals may either increase or decline across trials, phenomena called sensitization and desensitization, respectively. We tested if mustard oil (allyl-isothiocyanate) elicits a sensitizing or desensitizing pattern of oral irritation, and if it exhibits cross-desensitization with capsaicin. This was assessed by obtaining successive ratings (using a bipolar category scale) of the intensity of irritation elicited by sequential applications of mustard oil (0.125%, 40 μl) 10 times at 1-min intervals to one side of the tongue. After a 10-min break, either mustard oil or capsaicin (10 ppm, 20 μl) was applied bilaterally and in a 2-alternative forced choice (2-AFC) procedure subjects chose which side had more intense irritation and rated irritant intensity on both sides. Ratings significantly declined across trials (desensitization). In the 2-AFC subjects consistently chose the side not previously receiving mustard oil as more intense for both mustard oil and capsaicin, and assigned significantly higher intensity ratings to that side. Capsaicin exhibited sensitization and cross-desensitization of irritation elicited by mustard oil. Sequential application of mustard oil at faster (20 sec) intervals initially evoked a sensitizing pattern followed by desensitization. The temporal patterns of oral irritation exhibited by mustard oil, and its reciprocal cross-desensitization with capsaicin, are similar to those of menthol and nicotine.

ASSESSMENT OF OCULAR AND NASAL IRRITATION IN ASTHMATICS RESULTING FROM FRAGRANCE EXPOSURE

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Asthmatics often report hyper-sensitivity to odors and irritants found in many household and cosmetic fragranced products. Although asthmatics report trigeminally-mediated upper airway and ocular symptoms at lower thresholds than non-asthmatics, little evidence exists to determine whether such subjective reports correlate with objective changes in the upper airways following fragrance exposures. Subjective symptom reports were compared to objective measures in 157 asthmatics and 214 non-asthmatics following exposure to one of two fragrance mixtures and a clean air control. Measures of nasal mucosal swelling, via acoustic rhinometry, and photographic assessments of ocular hyperemia, using high-resolution macro-photography, were taken before exposure, after an initial 5-minute exposure and again after a 30-minute exposure. Both methods allow quantification of mucosal swelling or conjunctival redness after exposure to a variety of chemicals and are sensitive indices of localized irritation in upper airways and conjunctiva. Although, moderate asthmatics tended to report more nasal stuffiness following fragrance exposure than did non-asthmatics, no objective changes in ocular redness or nasal mucosal swelling were observed. Whereas some asthmatics may experience sensory irritation following exposure to different fragrance compounds, asthmatics may also (1) attend more closely to subtle changes in physiological substrates or (2) have a greater bias to report changes in physiological status when triggered by other sensory cues (e.g., odor). Supported by S.C. Johnson and NIH P50 DC00214

SENSORY PERCEPTION OF PARTICULATE MATTER FROM MINERALS

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Twelve young men participated in a study of chemesthesis and associated effects from exposures to dusts of calcium sulfate, sodium borate (sodium tetraborate pentahydrate), and calcium oxide. Size distribution of each dust peaked in the range 7-17 μ m mass median diameter. Exposure took place for 20 min at a time in a transparent dome that covered the head. The subject exercised at a level that simulated light work. Outcome variables included rated feel of the dusts in the eye, nose, and throat, nasal resistance, amount of nasal secretion, and time of mucociliary clearance. Feel increased at a negatively accelerated rate over time, with time-course associated with water solubility of the dust. The nose registered more feel than the throat and much more than the eye. For the strongly alkaline material calcium oxide, feel exceeded that of a blank (air) at an exposure of 1 mg/m³. For the less alkaline sodium borate, feel exceeded that of the blank at 5-10 mg/m³. For the relatively inactive material calcium sulfate, feel exceeded that of the blank at 40 mg/m³. Amount of nasal secretion, measured by a gain in weight of a sponge placed onto the septum, correlated with feel (r=0.88). Neither nasal resistance nor mucociliary clearance varied systematically with feel at the exposures employed. Exposure to 2 mg/m³ calcium oxide, a common occupational exposure limit for that material, caused a level of feel equal to that of 14-15 mg/m³ sodium borate and 46-47 mg/m³ calcium sulfate. The study implicates both physical and chemical factors in chemesthesis from mineral dusts. Supported by a gift from U.S. Borax Corp.

INDIVIDUAL DIFFERENCES IN NASAL LOCALIZATION FUNCTIONS FOR CARBON DIOXIDE

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Many studies of trigeminal function in the nose use carbon dioxide (CO₂) since it has little or no odor. However, basic psychophysical data on nasal detection of CO₂ are scarce. The current study addressed two basic questions. 1) What mathematical function best describes the psychometric function for nasal localization of CO₂? 2) Do substantial individual differences in sensitivity exist? While breathing through the mouth, 12 healthy subjects received 500 ms pulses of CO₂ diluted in air (15-40%, method of constant stimuli) in one nostril, and pure air in the other nostril. Subjects sought to determine which nostril received CO₂. Gaussian, logistic, and Weibull functions were fit to localization functions through maximum likelihood estimation. On average, two-parameter Weibull functions fit localization functions better than did Gaussian functions, which in turn fit better than logistic functions (X-squared = 57.9, 64.7, and 68.82, respectively, on 48 df). The average threshold for localization equaled 27.1%. Thresholds for individuals ranged from 19.8 to 34.4%. Ninety-five percent confidence intervals for individual thresholds indicated that many individual differences reached statistical significance. In short, a Weibull function fits nasal localization functions for CO₂ better than other sigmoidal functions, and substantial individual differences in sensitivity exist. Supported by NIH grants DC00014 and DC00214

DOES HABER'S LAW APPLY TO SENSORY IRRITATION?

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Toxicologists model the human health impact of airborne chemicals, including those producing sensory (eye, nose and throat) irritation. The two principal exposure variables in such modeling are concentration (c) and time (t), factors which are postulated to interchange under the terms of Haber's Law ($c \times t = k$, or more generally, $c^n \times t = k$; where k = constant for a given toxicologic endpoint). To evaluate the applicability of Haber's Law to sensory irritation, we systematically reviewed the human experimental literature for three chemicals (ammonia, chlorine, and formaldehyde), as well as searched for other relevant studies. We identified a total of four sensory irritation studies in which both concentration and time varied, making them applicable to evaluating Haber's Law. The agents employed in these studies included ammonia (1 study), chlorine (2 studies), and 1-octene (1 study). The simplest form of Haber's law ($c \times t = k$) predicted subjective nasal irritation by ammonia when exposure times were very brief (i.e., less than 4 sec.). With the substantially longer exposures involved in the remaining studies, however, higher concentration appeared to contribute more than prolonged duration to achieve a given subjective irritancy rating. Sufficient data were presented in one chlorine study to permit empirical evaluation of this effect. For throat irritation, the best fit between $[c^n \times t]$ and [proportion of subjects reporting a criterion sensory rating] was achieved with $n=2$ ($p<0.01$). Definitive evaluation of the applicability of Haber's Law to sensory irritation would require the completion of additional studies incorporating both multiple exposure levels and rating times. Results may vary by chemical compound and/or specific anatomical target.

UNDERGROUND CABLE GNAWING REPELLENT EFFECTS WITH CAPSAICIN TREATMENTS IN NORTHERN POCKET GOPHERS (T. TALPOIDES) AND PLAINS POCKET GOPHERS (G. BURSARIUS).

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Gnawing damage to buried communications and power cables by pocket gophers has been continually reported. Methods of reducing or preventing these animals from causing gnawing problems are needed and gaining in priority as repair and replacement cost rise. Field trials in areas inhabited with northern pocket gophers and in rangeland inhabited with plains pocket gophers have indicated repellent protection of cable samples inserted into their burrow systems. Repellent treatment consisted of a polybutene carrier and 2.0 percent active ingredient of capsaicin oleoresin contained within plastic tubing. Approximately 40 gophers were evaluated for gnawing damage prior to repellent treatment with untreated cable segments for 3 to 6 weeks of exposure. The same procedure was repeated with either the carrier-alone or carrier plus capsaicin treatment. For the trial with northern gophers, there was 85 percent reduction in damage for depth of gnaw damage and 77 percent reduction for the width of gnaw damage. A commercial capsaicin coating on the cables produced 34 percent reduction on damage width, but no effect on depth. For plains pocket gophers, overall damage was reduced by 43 percent, with the main effect of reduced width of gnaw damage. We concluded that a substantial level of gnawing can be prevented using the jacketed cable treatments in Western and Mid-Western areas of the USA where cables often traverse historical burrowing rodent habitats. Supported by USDA, Animal and Plant Health Inspection Service (Wildlife Services) funding.

IN VIVO EFFECTS OF CAPSAZEPINE ON TRIGEMINAL NERVE SENSITIVITY TO CARBON DIOXIDE AND NICOTINE

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Capsazepine (CPZ), a competitive inhibitor of vanilloid receptors (VR-1) has previously been shown to also inhibit nicotinic acetylcholine receptors (nAChR) in cultured rat trigeminal ganglion neurons, calling into question its reliability as a specific receptor blocking agent. To determine the reliability of using CPZ as a specific vanilloid receptor blocker in an *in vivo* model, we examined the effects of systemic CPZ on nasal trigeminal nerve responses to nAChR and VR-1 activating stimuli in anesthetized Sprague-Dawley rats. Multi-unit neural recordings were obtained from the ethmoid branch of the trigeminal nerve in response to vapor-phase nicotine (12.5 ppm) and carbon dioxide (50%). Nicotine has been shown to activate neuronal nAChRs present on trigeminal nerve endings, and carbon dioxide is believed to activate acid-sensitive receptors, including VR-1, via a carbonic anhydrase mediated intracellular acidification mechanism. Stimuli were delivered to the nares of the rats via an air-dilution olfactometer. Results indicate that CPZ (10⁻⁶ moles/kg) selectively decreases nerve response to carbon dioxide, whereas response to nicotine does not significantly change. These results suggest that at the concentration tested, systemic CPZ does not interfere with nAChR function, and can be reliably used as a specific vanilloid receptor blocker.

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF P2X-RECEPTORS IN CULTURED RAT TRIGEMINAL NEURONS

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Trigeminal nerve fibers innervating the facial mucous membranes (eye, nose, mouth) can perceive and discriminate between various chemical stimuli. Until now, little is known about the receptors and signal transduction pathways of the trigeminal system. m-RNA transcripts and proteins of purin receptors (P2X) have been identified in these neurons. In situ, trigeminal neurons express predominantly the subunits P2X2 and P2X3, which can form homomeric or heteromeric receptors. P2X receptors are ligand-activated ion-channels, activated by ATP, which is an important transmitter/ co-transmitter/ modulator and can play a functional role in the trigeminal system.

Using whole-cell voltage-clamp technique we investigated the response of cultured trigeminal neurons dissociated from rat *ganglion gasserii* to application of various concentrations of ATP. The dose-response-relationship showed an EC50 of 30µM ATP with a Hill-coefficient of 0.8. Three populations of neurons differing in the kinetics of the ATP-induced currents could be identified: phasic, tonic, phasic-tonic. Specific agonists and antagonists allowed to identify the subunit composition. The tonic currents were carried by homomeric P2X2 receptors, the phasic currents by homomeric P2X3 receptors and the phasic-tonic currents by homomeric P2X2 and P2X3 receptor populations. We never get any indication for heteromeric P2X2/3 receptors. In further studies we will investigate a possible role of ATP receptors in perception of chemical stimuli.

SIMILARITIES BETWEEN CAPSAICIN- AND METHYL ANTHRANILATE-SENSITIVE CHICKEN TRIGEMINAL NEURONS

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Birds are strongly repelled by methyl anthranilate (MA), but not by capsaicin (CAP). For mammals the reverse is true; CAP is a potent irritant. Cultured chick trigeminal (TG) neurons do in fact respond to both CAP and MA, as determined by fluorescence imaging of [Ca2+]i. To determine if there are different populations of CAP- and MA-sensitive neurons in chick TG ganglion that mediate the differential behavioral response to these compounds, we examined the size distribution and vanilloid (CAP) receptor 1-like immunoreactivity (VR1-like IR) in neurons responsive to 30 µM CAP or 30 µM MA. With the exception of a few large CAP-sensitive neurons, the size distributions of CAP- and MA-sensitive and insensitive neurons were not different (p>.05, chi-square test). This is different from mammals in which CAP-sensitive neurons are small. VR1-like IR in chick TG neurons is not restricted to a specific functional class nor size of neurons. To further characterize chemical sensitivity in chick neurons, capsazepine (CPZ), an antagonist of CAP in mammals was used. CPZ did not antagonize responses to either CAP or MA in chick TG neurons. Rather, CPZ caused an increase in [Ca2+]i in some neurons. Sensitivity to CPZ partially overlapped with sensitivity both to CAP and to MA, indicating that these three agents activated separate transduction mechanisms. Although there are differences in the distribution of sensitivities to CAP and MA, neither neuronal somatic size nor immunoreactivity to a potential receptor mechanism distinguishes between the different functional classes of neuron. This research was funded in part by USDA/National Wildlife Research Center.

BEHAVIORAL EFFECTS OF STATIC MAGNETIC FIELDS ON FREELY MOVING AND RESTRAINED MICE

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High strength static magnetic fields are becoming common tools in clinical imaging, although the behavioral effects on subjects are not well known. Previous studies on rats indicate that higher strength fields, 7 T or above, produce acute locomotor circling activity, conditioned taste aversion and c-Fos activation in the vestibular nuclei. In the present study we subjected mice to a 30 minute exposure to a 14.1 T magnetic field. Mice were placed in one of two conditions; restrained or freely-moving. Mice were given 30-min access to saccharin immediately prior to 30-min magnet exposure or sham exposure on three consecutive days. Mice were evaluated for locomotor activity and acquisition of a CTA following magnet or sham exposure. All mice exposed to the magnet developed a CTA, while a significant number of mice displayed tight circling and suppression of rearing. Mice in the freely-moving condition exhibited larger effects when compared to restrained or sham-treated mice. These effects may be the result of a vestibular disturbance caused by the magnetic field exposure. Supported by NIHDCD 04067 and NIH Joint Neuroscience Predoctoral Training Grant NIA, NICHD, NIDCD, NIDCR, NIGMS, NIMH, NINDS and NINR)T32 NS 7437.

CONDITIONED TASTE AVERSION INDUCED BY HIGH MAGNETIC FIELDS IN MALE AND FEMALE RATS DEPENDS ON POSITION WITHIN THE FIELD

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When intake of a palatable solution is paired with exposure to high strength magnetic fields, rats acquire a conditioned taste aversion (CTA). This indicates that rats can detect a magnetic field. However, the mechanism and parameters required for field detection are not known. In this experiment, we varied the position of rats within a 14T superconducting magnet and measured subsequent CTA expression. Male or female rats on a water-deprivation schedule were given 10 min access to 0.15% saccharin and then restrained within the superconducting magnet. Rats were stacked vertically 5 at a time within the magnet (n=5-6/position), such that rats were positioned at 30cm intervals from the bottom of the bore; the 2nd and 3rd rats from the bottom overlapped with the peak intensity of field (14T). After 30 min, rats were removed from the magnet and returned to their home cage. 24 h later, 2 bottle 24h saccharin:water preference tests were begun and continued for 14 days. Male rats in the 2nd position expressed a profound and long-lasting saccharin aversion; all other positions induced only weak or no aversions. Female rats in the 1st, 2nd, or 3rd positions expressed significant saccharin aversions. We conclude that the exact position of the rat within the magnet is critical to the acquisition of a CTA. Furthermore, it appears that female rats are more sensitive to the effects of the high strength magnetic field than male rats. Supported by NIDCD04607.

TASTE PRE-EXPOSURE ATTENUATES BOTH BEHAVIORAL AND NEURAL EXPRESSION OF A CONDITIONED TASTE AVERSION IN RATS.

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While learning which foods are toxic is critical to survival, learned safety about familiar foods is also an important feature of taste learning. In our experiment, we tested the behavioral acquisition of a CTA against intraoral infusions of sucrose and the conditioned c-Fos induction in the NTS in rats with ten days prior exposure to sucrose vs. rats with no previous exposure. Rats were given 10 days ad lib access to 5% sucrose (n=6) or water (n=4). All rats were then implanted with intraoral catheters and food-deprived 24 h prior to each of three pairings of an intraoral infusion (6ml/ 6 min) of sucrose solution (5%) paired 30 min later with a systemic injection of LiCl (0.15 M, 12ml/kg). Intake was measured as the increase in body weight during the infusion. 48 h after the 3rd pairing, the rats were given a final unpaired intraoral infusion of 5% sucrose; 1 h later the rats were perfused and their brainstems processed for c-Fos immunohistochemistry. When examined behaviorally, intakes were higher in rats with pre-exposure to sucrose than in rats with no pre-exposure during both the 2nd and 3rd pairings of sucrose and LiCl. When the NTS was examined for c-Fos induction, the rats with pre-exposure showed significantly less c-Fos than the rats with no prior exposure (102 +/- 16 vs. 150 +/- 8, p<0.05). Thus, an intraoral CTA was learned more slowly in rats with prior taste exposure, and pre-exposure also decreased the conditioned neural response of the NTS after CTA expression. Supported by NIDCD 03198.

BEHAVIORAL AND NEURAL EXPRESSION OF CONDITIONED CAPSAICIN AVERSION

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Capsaicin stimulates trigeminal somatosensory nerves rather than gustatory nerves. It is not clear whether rats learn an aversion to capsaicin through the same mechanism as a gustatory conditioned taste aversion (CTA). Here we determined expression of a behavioral aversion with rats conditioned against intraoral capsaicin paired with LiCl. To test the neural expression of capsaicin aversion, we examined conditioned c-Fos in the intermediate nucleus of solitary tract (iNTS). Rats were implanted with intraoral catheters and received 3 pairings of an intraoral infusion (6ml/ 6min) of capsaicin (0.01 mM) paired 30 min later with LiCl or NaCl (0.15 M, 12ml/kg) when water-deprived. Between pairings, rats were infused with vehicle (0.01% Tween 80 & 0.01% EtOH) without LiCl so that rats only received contingent experience of vehicle when capsaicin was present. Two days after the last pairing, rats received a final, unpaired intraoral infusion of capsaicin. Rats were perfused 1 h later and the brainstem processed for c-Fos IHC. In the final infusion, LiCl-paired rats rejected all of the infused capsaicin, while NaCl-paired rats drank 3.2 ± 1.1 g. Thus capsaicin paired with LiCl induced a significant capsaicin aversion ($p < 0.05$). When the iNTS was examined, capsaicin induced significantly more c-Fos in LiCl-paired rats than in NaCl-paired rats (53 ± 17 vs 18 ± 3 , $p < 0.05$). We conclude that although capsaicin is detected by somatosensory nerves rather than gustatory nerves, the behavioral and neural integration of capsaicin during aversion learning is similar to that of true gustatory CTA. Supported by NIDCD 03198.

TASTE-POTENTIATED ODOR AVERSIONS: EFFECTS OF EXCITOTOXIC LESIONS OF THE AMYGDALA, VENTROPOSTEROMEDIAL THALAMIC NUCLEUS AND INSULAR CORTEX IN RATS

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The present study examined the contribution of the mediodorsal thalamic nucleus (MD), orbitofrontal cortex (OFC), parvocellular part of ventroposteromedial thalamic nucleus (VPMpc), insular cortex (IC), and amygdala in the formation of taste-potentiated odor aversions (TPOA). Rats with excitotoxic lesions of these structures and nonoperative control group were trained to acquire TPOA by presenting a saccharin solution flavored with isoamyl acetate (conditioned stimulus, CS), which was followed by delayed lithium chloride administration (unconditioned stimulus, US). Subsequent tests with isoamyl acetate alone showed that amygdala-lesioned group were impaired in the acquisition of odor aversions, while VPMpc- and IC-lesioned groups acquired odor aversions, but with rapid extinction. The tests with saccharin alone showed that VPMpc-, IC- and amygdala-lesioned groups failed to acquire taste aversions. MD- and OFC-lesioned groups acquired strong odor and taste aversions. These results suggest that the amygdala is involved in the establishment of aversions to odor and taste in TPOA, whereas the VPMpc and IC are concerned with aversions to only taste in the TPOA paradigm. The disruption of taste aversion by lesion of the VPMpc and IC lesion might be related to the attenuation of the taste-potentiated odor aversions. Supported by Grants-in-Aid for scientific research (Nos. 13710035 to TI and 11557135 to TY) from the MEXT of Japan, and by JSPS-RFTF97L00906 to TY.

RESPONSES TO HOST ODOR BY THE LIMULUS WORM

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The Limulus Worm (*Bdelloura candida*) is a 3-20 mm turbellarian flatworm commensal on horseshoe crabs (*Limulus polyphemus*). Worms move upstream in currents containing *Limulus* odor, but not in currents containing odor of sympatric crustacea. We hypothesize that detection of host odor plays a role in searching out the host when it molts or finding a new host if the original host dies. In a 17 cm flume with 0.23 cm/s background flow we tested responses of single worms to three dyed stimuli: raw seawater, food odor and odor from live horseshoe crabs. Worms were placed downstream and videotaped for 10 minutes. Each of 10 worms was tested with all three stimuli. All paths were digitized and used to calculate distance covered, idle time, crawling speeds, heading angles, and turning angles. Worms covered significantly more distance in *Limulus* odor than in food odor ($P < 0.05$) or seawater ($P < 0.001$), but no difference was found between food odor and seawater. Worms spent significantly less time sitting idle in *Limulus* odor than in seawater ($P < 0.005$), but no difference was found between *Limulus* odor and food odor or between food odor and seawater. The results suggest that worms search most actively for *Limulus* odor. In seawater plumes, worms never came closer than 4 cm from the source and their crawling speeds, heading angles, and turning angles were highly variant. As worms approached the source in food or *Limulus* plumes, their turning angles remained roughly constant, but their speeds decreased and their heading angles increased, suggesting that the worms may use odor-gated rheotaxis to search for the source. Supported by the Boston University Marine Program.

SENSORS AND MOBILE PLATFORM FOR ELECTRONIC OLFACTION

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We are developing a new class of odor sensors to enable a mobile robot to locate airborne odor sources. The odor sensors are organic thin-film field-effect transistors fabricated from organic semiconductor materials. We have tested more than 15 different oligomers and polymers, such as α -sexithiophene and its di-butyl, di-hexyl, di-dodecyl and di-octadecyl congeners for responses to more than 16 different odorants, including alcohols, ketones, nitriles, esters and ring compounds. Odors were puffed onto the exposed active semiconductor region of the device and the source-drain current recorded with and without odor application. We find that several of the semiconductor materials are sensitive in the 10-100 ppm range and can give reproducible responses to more than 70 successive odor stimuli. Incorporation of these sensors into active circuits incorporating design principles from biological olfaction should greatly increase their sensitivity. A mobile robot has been fitted with a commercial enose (Cyranose 320) so that sensor responses can be obtained via a wireless link used to obtain visual and auditory signals from the robot and provide commands to its driver motors. Algorithms are being developed to utilize periodic odor sensor readings and information on wind direction to make course corrections and localize an odor source. Supported by NIMH grant MH-56090 to A.G.

FERTILIZATION IN THE SEA: THE CHEMICAL IDENTITY OF AN ABALONE SPERM ATTRACTANT

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Chemical communication between sperm and egg is a key factor mediating sexual reproduction. Dissolved signal molecules that cause sperm to orient and accelerate towards an egg could play pivotal roles in fertilization success, but such compounds are largely undescribed. This investigation considered the behavioral responses of red abalone (*Haliotis rufescens*) sperm to soluble factors released into seawater by conspecific eggs. Sperm in proximity to isolated live eggs swam significantly faster and oriented towards the egg surface. Bioassay-guided fractionation was employed to isolate the chemoattractant, yielding a single pure, fully active compound after reversed-phase and size-exclusion HPLC. Chemical characterization by NMR indicated that the free amino acid L-tryptophan was the natural sperm attractant in *H. rufescens*. L-tryptophan was released by eggs at concentrations that triggered both activation and chemotaxis in sperm, exhibiting significant activity at levels as low as 10^{-8} M. The D-isomer of tryptophan was inactive, showing that the sperm response was stereospecific. Serotonin, a potent neuromodulator and tryptophan metabolite, had no effect on sperm swim speeds or orientation. In experimental treatments involving either an elevated, uniform concentration of tryptophan (10^{-7} M) or the addition of tryptophanase, an enzyme that selectively digests tryptophan, sperm failed to navigate towards live eggs. A natural gradient of L-tryptophan was therefore necessary and sufficient to promote recruitment of sperm to the surface of eggs in red abalone.

THE EFFECT OF ODOR PULSE FREQUENCY ON THE ORIENTATION BEHAVIOR OF THE CRAYFISH, *ORCONECTES RUSTICUS*.Kozlowski C.P.¹, Moore P.A.¹ ¹*Biological Sciences, Bowling Green State University, Toledo, OH*

Crustaceans use chemical signals for many purposes, including communication, and locating food, mates, and shelter, but the mechanisms by which crustaceans locate odor sources in turbulent environments are not fully understood. Chemical signals are shaped by the fluid medium in which they are found, and it is these fluid conditions that determine the signal's spatial and temporal distribution. Under laminar flow, a plume forms with distinct boundaries and gradients. However, laminar flow is rare in nature, and turbulence and plume meandering give rise to intermittency in the chemical signal. To use chemical signals effectively, organisms must employ orientation strategies that allow them to overcome signal intermittency. In this study, we presented crayfish, *Orconectes rusticus* to odor plumes with a controlled increase in intermittency. Crayfish were exposed to odor plumes formed by a continuously releasing jet, or to odor pulsed at 0.5, 0.66, 1, 2, and 3 Hz in a re-circulating flume. Trials were videotaped from above and digitized at one frame per second for analysis. Results indicated that the orientation behavior of crayfish to pulsed odor plumes was significantly different from behavior to a continuously releasing jet. Crayfish walked straighter, faster, had more accurate heading angles, and took less time to find the source in a continuous odor plume than in plumes of pulsed odor. However, there was no significant difference in the success rate in the 1, 2, and 3 Hz plumes when compared to the continuous plume, indicating that crayfish are able to locate an odor source in a pulsed odor plume but do so less efficiently.

UNILATERAL LESIONING OF CHEMORECEPTORS DISTINGUISHES BETWEEN RHEOTAXIS AND CHEMOTAXISKraus-Epley K.E.¹, Moore P.A.¹ ¹*Biological Sciences, Bowling Green State University, Bowling Green, OH*

The goal of this project was to determine whether crayfish are orienting using a mechanism of chemotaxis, similar to lobsters, or odor-gated rheotaxis, which is used by blue crabs. A true chemotaxis is orientation purely mediated by the chemical signal itself and stimulation of the chemoreceptors. Conversely, odor-gated rheotaxis is orientation guided by the structure of the odor plume and the flow direction of the fluid medium, using the chemoreceptors and mechanoreceptors, respectively. Therefore, by unilaterally lesioning only the chemoreceptors, we can determine whether these animals are orienting using chemotaxis or odor-gated rheotaxis. In theory, if the animals can successfully orient to an odor source with unilateral lesions of chemoreceptors and bilaterally intact mechanoreceptors, it is likely that odor-gated rheotaxis is being employed instead of chemotaxis. Chemoreceptors on crayfish antennules were lesioned by placing the animals in a saltwater bath for 48 hours and subsequently bathing the antennules in deionized water for 5 minutes. After a reacclimation to freshwater for 24 hours, the animals were used in an orientation trial where fish gelatin was the food source. Orientation video was digitized and orientation parameters were analyzed. The results of this study demonstrate which receptors, chemo- and/or mechanoreceptors, are necessary for successful orientation and give us insight into which orientation mechanism crayfish may be using to orient.

ORIENTATION IN COMPLEX SENSORY LANDSCAPES: SPATIAL ARRANGEMENT OF ODOR SOURCES MODIFIES ORIENTATION STRATEGIES OF CRAYFISH.Wolf M.C.¹, Moore P.A.¹ ¹*Biological Sciences, Bowling Green State University, Bowling Green, OH*

In natural aquatic systems organisms are presented with multiple food cues that are distributed in space and have to make decisions on how to respond to this plethora of information. Previous orientation work has relied on using one food odor stimulus where in natural habitats they are bombarded with multiple food information that can impact foraging decisions. The present study investigated how the spatial distribution of multiple food cues can affect crayfish orientation. Crayfish *Orconectes virilis* were presented with a series of food cues that were differentially spaced within an artificial stream. Orientation behavior was filmed from above and digitized one frame per second. In addition, electrochemical recordings were taken to characterize the structure and distribution of the odor plumes. Finally, an acoustic doppler velocimeter (ADV) was used to characterize the hydrodynamic structure of the artificial stream. Crayfish showed significantly altered orientation strategies when presented with different spatial arrangements of food cues. Since altering the spatial arrangement of the odor sources does not impact the hydrodynamics, any changes in orientation strategies indicates a chemotactic strategy as opposed to a rheotactic strategy. This research was funded by NSF DAB, NSF Sensory Systems, and a BGSU TIE grant to PAM, and the University of Michigan Biological Station.

AMERICAN LOBSTERS TRACK AND LOCATE DISTANT "LEAKY" ODOR SOURCESVoigt R.¹, Basil J.², Atema J.³ ¹*Department of Biological Sciences, Bowling Green State University, Bowling Green, OH;* ²*Department of Biology, Brooklyn College, Brooklyn, NY;* ³*Boston University Marine Program, Boston University, Woods Hole, MA*

The spatial and temporal distribution of aquatic odor plumes is shaped by environmental conditions. Turbulent odor dispersal causes directional parameters of the patch distribution that vary with distance from an odor source. Lobsters may be using an odor-gated rheotaxis, i.e., the mean current for orientation once a chemical signal is detected, or eddy-chemotaxis, i.e., the internal chemical and hydrodynamic fine structure of an odor plume to locate the source. Lobsters were tested at distances of 4 or 7 m from the source in a recirculating flume (mean flow rate 5 cm/s). Stimuli were delivered as a low momentum 'leaky' plume. Walking speeds and heading angles were used to quantify an orientation path. Far from the source larger heading angles and slower walking speeds were observed. As lobsters approached the odor source, their headings improved and walking speeds increased suggesting that a spatial gradient within the plume had been found for tracking. Close to the source, lobsters walked more slowly. We observed no apparent differences in tracking behavior with different starting distances. These results favor the hypothesis that lobsters follow an internal plume gradient, which becomes easier to follow closer to the source. This gradient could be based on chemical and/or hydrodynamic components. It is less likely that odor-gated rheotaxis is used since mean flow does not vary with distance. Acknowledgement This research was supported by a grant from the Office of Naval Research (N000-1498-10-822, Keith Ward, Program Officer) to JA

DIFFERENT POPULATIONS OF ANTENNULAR CHEMOSENSILLA CAN MEDIATE THE ORIENTATION OF SPINY LOBSTERS

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The antennules of the Caribbean spiny lobster (*Panulirus argus*) contain a diversity of chemoreceptive sensilla. Chemoreceptor neurons associated with aesthetasc sensilla project to the glomerular olfactory lobes, whereas those associated with non-aesthetasc sensilla project to the stratified lateral antennular neuropils. There are thus two parallel pathways for chemosensory input from the antennules; but what is the function of each pathway for odor mediated behaviors? This study investigates the importance of the two pathways for orientation by determining whether aesthetasc or non-aesthetasc sensilla are necessary and sufficient for a lobster to locate a distant odor source. The study was conducted in a seawater flume using flow rates of 5 cm/s. Lobsters were placed 2m downstream of an odor stimulus and their paths to the source were recorded and analyzed. To assess the importance of aesthetasc versus non-aesthetasc sensilla, we performed a series of selective ablations that removed specific populations of antennular sensilla and compared the behavior of ablated animals to intact controls. Results indicate that while both aesthetascs and non-aesthetascs are sufficient for orientation, neither population is necessary under the conditions tested. Although ablated animals performed less efficient searches than intact controls, there was no difference in search efficiency between groups with aesthetascs or non-aesthetascs removed. Thus there appears to be some redundancy in the function of the two chemosensory pathways during orientation under the current conditions. Supported by NSF IBN-0077474, NIHDC00312, and the Georgia Research Alliance

AN EVALUATION OF FACTORS RELATED TO THE DETERMINATION OF THE DIRECTION OF QUARRY MOVEMENT BY EXPERIENCED TRACKING DOGS

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Although dogs are often used to track individuals, there is some question as to whether a dog can approach the track at a right angle and travel in the direction taken by the track-layer. In our study, 28 trained scent-following dogs were given from 4 to 20 trials while leashed. On each, the dog approached the midpoint of a track (usually 92 meters long) at a right angle and traveled to either end. The correct response was defined as traveling in the same direction as the track-layer; only two dogs reliably exhibited this ability. When results for all dogs were combined, chi square tests showed that: i) the starting direction, which was no more likely to be the correct one, does predict the final destination; and ii) the likelihood of the dog choosing the correct direction was unrelated to the number of reversals. These results, considered with prior evidence that off-leash dogs can discern the direction the track-layer traveled, suggest that this ability is greatly diminished when the dog is tethered to the handler.

MODIFICATION OF THE SILKMOTH PHEROMONE-SEARCHING BEHAVIOR BY VISUAL INFORMATION, CIRCADIAN RHYTHMS AND SEROTONIN

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The pheromone-searching behavior of male silkmoths is influenced by external (visual information) and internal cues (serotonin and circadian rhythms), which are correlated to each other. Behavioral experiments consisted in behavioral threshold experiments and in recording and analyzing different parameters of the movements of the moth on a ball connected to a computer in response to synthetic pheromone. We also performed intracellular recordings with Lucifer Yellow staining of protocerebral neurons as well as high performance liquid chromatography with electrochemical detection of biogenic amines. The behavior was fully observed in daytime and light conditions ("high state"), while in daytime but dark conditions, or nighttime (light or dark), the behavior was reduced ("low state"). Single neuron results suggested that visual neurons, in light conditions, might have a modulatory effect on other types of neurons (olfactory, mechanosensory). Circadian variation of serotonin in the brain (4 am vs. 8 pm, ANOVA, $P < 0.05$) could explain changes of responses to pheromone at the single neuron and behavioral level. This hypothesis is supported by the fact that injecting serotonin in the male silkmoth brain increased the sensitivity to pheromone (Fisher test, $P < 0.05$) while injecting serotonin-antagonists had the opposite effect (Fisher test, $P < 0.05$). Supported by PROBRAIN and the Ministry of Education, Culture, Sports, Science and Technology of Japan

MEASURING CANINE OLFACTORY FUNCTION NATURALISTICALLY

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Though the canine olfactory system is heavily relied upon to strengthen decision-making in many areas, quantitative data on this "instrument" are largely confined to a few odor thresholds. These values derive from laboratory procedures bearing little relation to behavioral methods underlying success of dog-handler teams in field settings. As a first step in addressing the need for more and better information, we selected four dogs with much prior training (AKC UD level) and used retrieval and search procedures to associate praise reward with localization of the odor of n-amyl acetate (nAA). Searches were then narrowed to four (initially wood and then Teflon) boxes, one of which contained nAA at ~ 3ppb. After each dog sat reliably, in repeated blind trials, in front of only the box with the nAA stimulus the procedure was moved from field to laboratory. With concentrations ranging from ~3.84 to ~0.06 ppb presented over the course of 33 trials for each dog, the number of correct responses varied from 28 to 33. This method is now being employed for threshold determinations and is suitable for testing of much more complex olfactory functions.

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MODELING OF HUMAN OLFACTORY ADAPTATION

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Olfactory adaptation and recovery is defined as the decrease and recovery of the olfactory sensation (sense of smell) during and after prolonged or repetitive exposure to odorants. This process is essential for human perception or detection of an odor in everyday life. Substantial variations in this process may also play an important role in olfactory dysfunction. We developed a lumped parameter mathematical model of human olfactory adaptation based on mucosal odorant flux in the human nose. An anatomically accurate, 3-D odorant mass transfer finite element model of the human nasal cavity, refined by comparison with experimental human nasal absorption data, serves as input to the model. Other physiological and physicochemical inputs include: nasal submucosal blood flow rate, odorant concentration in the blood, olfactory mucosal thickness, and odorant physio-chemical properties in olfactory mucus. Preliminary results of our model are consistent with olfactory adaptation data for both threshold and supra-threshold responses and suggest that submucosal blood flow rate (whose magnitude can be estimated from the model) and odorant mucus solubility are major parameters in controlling the time course of olfactory adaptation and recovery. Supported by NIH grant (NIH- P50 DC 00214)

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CONCENTRATION MODULATION OF SNIFFING IN HUMANS REVEALS RAPID OLFACTORY PROCESSING

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Sniffs are modulated in response to odor content. Higher concentrations of odor induce lesser-volume sniffs. This phenomenon implicates a neural feedback mechanism that measures sensory input (odor concentration) and modulates motor output (sniffing) accordingly. Here we used air-dilution olfactometry to probe the time course of this mechanism. A stainless-steel computer-controlled olfactometer, equipped with mass flow controllers, temperature and humidity control, and on-line photo-ionization detection was coupled to a highly sensitive pneumotachograph that measured nasal flow. The olfactometer was used to generate 5 ascending concentrations of the odorants amyl acetate, propionic acid, and vanillin. To date, 7 young healthy subjects participated in the study. As previously shown, sniff volume was inversely related to odor concentration ($r = .4$, $F(1,272) = 46$, $p < .0001$). Preliminary analysis of the concentration-specific sniffgrams suggests that a sniff may adopt a concentration-dependant slope from as early as 300 ms. following sniff onset. Considering that odorant transduction may take around 150 ms. and odorant-induced cortical evoked potentials may have latencies of around 300 ms., the time course measured here suggests that sniff feedback control is achieved through very few synapses, and may be subcortical. Funding provided by the Searle Fellowship, NIH-NIDCD, and SOSI

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DOSE-DEPENDENT RESPONSES OF MANDUCA SEXTA "PHEROMONE-SPECIFIC" OLFACTORY NEURONS TO GENERAL ODORANTS

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The olfactory receptor neurons (ORNs) in the sexually dimorphic trichoid sensilla of male *Manduca sexta* are exquisitely sensitive to components of the female's pheromone blend. Traditionally, these ORNs are regarded as specialist cells because of their narrow receptive field. In contrast, ORNs of basiconic sensilla are believed to detect a much wider range of odorants, including plant volatiles. We performed single-unit recordings of ORNs within male type-I trichoid sensilla and monitored their responses to a broad panel of 85 non-pheromonal odorants. Surprisingly, at moderately high concentrations ~30% of these odorants consistently elicited repeatable, concentration-dependant responses. Two major classes of excitatory odorants were identified: long chain, unbranched aldehydes and branched chain esters. ORN responses varied predictably within an odorant class with respect to carbon chain length, degree of unsaturation, and functional group. Interestingly, odorants recently reported as excitatory to the female type-A trichoid sensilla (Shields & Hildebrand, 2001) produced small or negligible responses in male type-I trichoid sensilla. Likewise, odorants which strongly activated the male ORNs were marginally stimulatory in the female. It remains to be seen how this capacity of type-I trichoid sensilla ORNs to detect a more diverse set of odorants, though at a lower sensitivity, influences integration and processing in the olfactory lobe and how this information is in turn translated into behavior

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DISRUPTION OF GAP JUNCTIONS IN OLFACTORY NEURONS ALTERS OLFACTORY RESPONSES TO SOME ODORS

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One of the challenging questions in understanding olfactory transduction is whether olfactory information is modulated in the primary olfactory pathway before transmission to the olfactory bulb. Our studies have demonstrated spatial distribution and expression of multiple gap junction protein subunits (connexins) in the olfactory epithelium. We hypothesize that gap junctional communication between olfactory neurons modulates peripheral olfactory coding. To test this hypothesis, we have designed and generated a dominant negative connexin 43 (Cx43) mouse (DNCX). The transgene includes a 0.9kb mouse olfactory marker protein (OMP) promoter that drives the expression of Cx43/-galactosidase fusion protein (Cx43/-gal). Cx43/-gal inactivates Cx43 channels by competing with endogenous Cx43 during assembly. Integration of the transgene was confirmed by Southern analysis. *In situ* hybridization showed that Cx43/LacZ mRNA was expressed in the olfactory epithelium and vomeronasal organs. The expression pattern was consistent with expression directed by the OMP promoter. Western analysis indicated the presence of Cx43/-gal in the olfactory epithelium. The ratio of electroolfactogram responses to octanal/benzaldehyde ($p < 0.001$, $n = 25$) and octanal/cineole ($p < 0.05$, $n = 19$), but not the ratio of cineole/benzaldehyde, were reduced in DNCX. Our study suggests that gap junctions are involved in modulating olfactory activity in some olfactory receptor neurons. This work was supported by grants DC00566 and DC04657 from the NIDCD.

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COMPLEX ELECTROPHYSIOLOGICAL RESPONSES OF CATFISH OLFACTORY RECEPTOR NEURONS TO AMINO ACID STIMULI

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Spontaneously active olfactory receptor neurons (ORNs) of brown bullhead catfish recorded *in situ* responded to amino acids. In a dose-response study (N=60), 32 ORNs responded with suppression (increased duration of initial interspike intervals) and 8 responded with excitation (increased duration of the maximum firing frequency). Cumulative Slope Analysis (Blejec, 2000) revealed four response types: A) concentration-dependent suppression (16 cells); B) concentration-independent suppression (16 cells); C) concentration-dependent excitation (3 cells) and D) concentration - independent excitation (5 cells). Of 52 ORNs tested with five amino acids and a mixture of 15 amino acids that included the five individually tested amino acids, 36 ORNs responded to at least one of the mixture components. Nine of these ORNs responded excitedly to some and were suppressed by the other amino acids. The 15 amino acid mixture triggered suppression in 25 ORNs and excitation in 2 ORNs. Sixteen of thirty-six ORNs that responded to at least one of the mixture components did not respond to the mixture, suggesting mixture suppression. Keeping the electrode at the position of the first spontaneous activity recording (N=40 ORNs) a second spontaneous activity was observed within 30 minutes of the silent period. The observation of the second spontaneous activity indicates that ORNs have active and inactive periods. The neuron's responsiveness to amino acid stimuli was retested and its identity determined by comparison of the amino acid responses before and after the silent period. Supported by MZT grant P0-0509-0487-01.

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IN SITU CALCIUM IMAGING FOR SPATIAL ODOR MAPS IN THE MOUSE OLFACTORY EPITHELIUM

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Despite increasing information on odorant-receptor interactions and the molecular architecture of odor signal inputs into the olfactory bulb, we have little knowledge about the spatial distribution of olfactory receptor neurons that respond to specific odorant molecules in the olfactory epithelium. We have developed a calcium imaging system that allows for the detection of odorant responses *in situ* in an intact coronal slice preparation of the mouse olfactory epithelium. Increasing odorant concentrations resulted in increases in the numbers of odorant-responsive neurons, supporting the evidence that a single odorant molecule is recognized by multiple receptors that have differing dose-response properties. Analyses of zonal distributions of the odor-responsive cells revealed the presence of regional localizations of the receptors. Different odorants exhibit different response profiles both in the numbers and distributions of responsive cells in the receptor expression zones, suggesting that there are odorant-specific spatial activity maps, and that the profiles are partially dependent on the type of functional groups in the odorant molecules. This method allows for visualization of spatial odor maps in anatomically intact slices of the olfactory epithelium, which add an important intermediate level of organization in the architecture of odorant reception.

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COMBINATORIAL PHEROMONE CODING VISUALIZED IN THE MOUSE MAIN OLFACTORY EPITHELIUM

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Studies directed at understanding pheromone sensing in mammals have focused on the vomeronasal organ (VNO), while there is considerable evidence that the main olfactory system sometimes interacts with the VNO to mediate neuronal responses to pheromone-like signals. Here, we have conducted a systematic analysis of the sensitivity and specificity of olfactory sensory neurons (OSNs) in the mouse main olfactory epithelium (MOE) to the same ligands that were previously shown to be potent activators of vomeronasal neurons (VNs) (Leinders-Zufall et al. 2000, *Nature* 405:792). Applying a combination of EOG recordings in intact MOE and patch clamp and confocal Ca²⁺ imaging methods in coronal MOE slices, we find that all ligands used previously in VNO activate also distinct subpopulations of OSNs. OSNs are surprisingly sensitive to these ligands, with threshold responses in the picomolar range. But unlike VNs, individual OSNs can respond to several of these ligands, even at very low concentrations, and cellular tuning curves do broaden with increasing concentrations of ligands. We map the representation of pheromones in the MOE with single-cell resolution and identify preferential detection zones for some of the ligands. Our results establish OSNs in the mouse MOE as surprisingly sensitive pheromone detectors and emphasize further that the principles for processing of chemical information in MOE and VNO are fundamentally different. Supported by NINDS and NIDCD (M.V.N, F.Z. and T.L.-Z.).

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MAPPING OLFACTORY RESPONSES TO CO₂ IN ADULT RATS

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Recent evidence suggests that a subset of olfactory receptors in adult rat nasal epithelium are sensitive to CO₂ concentrations as low as 2%-14%. The objective of the present study was to characterize the distribution of the electro-olfactogram (EOG) response to CO₂ over a large part of the nasal epithelium. This study involved the use of two similar, but separate protocols. The first involved eight male Sprague-Dawley rats overdosed with nembutal and prepared for recording from the olfactory epithelium according to previously described methods (Scott et al., 1997). Simultaneous recordings were made from four saline-filled glass electrodes in each rat. The electrode array was placed in locations that roughly corresponded to the four receptor-gene expression zones. CO₂ responses were present in all four zones of endoturbinate II' with a slightly larger response seen in the dorsal region of this endoturbinate. The second protocol involved a spatial mapping with a single EOG electrode (N=15, 240 sites). Responses were similar to those found in the four-electrode array in that the largest EOG amplitudes were seen in the dorsal region of each endoturbinate. These results comply with the regional distribution of CO₂ reactive cells seen through carbonic anhydrase staining (Coates, 2001). The waveforms characteristics of these responses are similar to those recorded previously (Cecala et al., 2001).

THE CONTRIBUTION EACH NOSTRIL MAKES TO OLFACTORY PERCEPTIONDe Puy K.S.¹, Hornung D.E.¹ ¹*Biology Department, St. Lawrence University, Canton, NY*

Utilizing the cyclic changes in the relative size of the airflow passageway for the two nostrils, Sobel (Nature 402:35, 1999) observed that with an octane/carvone mixture the nostril with the higher airflow was more sensitive to carvone, the highly sorbed odorant. The present study was designed to determine if this phenomenon occurs for other odor pairs and then to determine how the central nervous system deals with disparate information from the two nostrils. Using isointensive mixtures of carvone/octane, pentanol/butyric acid and isopropanol/hexanoic acid subjects used single nostrils to rate the contribution each component made to the intensity of the mixture. After the single nostril ratings, subjects made the same determination using both nostrils. Although The Sobel effect was observed for all odorant pairs, the nostril difference was less dramatic for the pentanol/butyric acid mixture. Two models seemed to be equally effective at predicting the binasal response from the responses of the individual nostrils. In the first model, the intensity ratings from the right nostril in right-handers and the left nostril in left-handers were a good predictor of the binasal response. In the second model an average of the intensity ratings from the two individual nostrils was also an excellent descriptor of the binasal response. In conclusion, although nasal anatomy has again been shown to influence individual nostril sensitivity, the central nervous system is able to meld these differences into a single sensation.

SPECIFIC EFFECT OF ODOR BUT NOT VISUAL IMAGERY ON DETECTION OF WEAK ODORSDjordjevic J.¹, Boyle J.¹, Zatorre R.¹, Jones Gotman M.¹
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We previously explored odor imagery in 24 subjects using an odor detection paradigm requiring detection of weak (threshold) odors while subjects imagined either the target odor (matched) or a different odor (mismatched odor imagery). We observed significantly better detection during matched odor imagery. We now pursue those results with a follow-up study addressing two questions: is the effect of odor imagery on odor detection specific, and what is the mechanism of this effect (interference or facilitation). To answer these questions, we tested 48 more subjects in one of two conditions: 24 were tested in a paradigm that was identical to our previous one except that it used visual instead of odor imagery (visual imagery condition). Subjects received 100 detection trials (PEA/Citral or a blank), during which they visually imagined either a rose or a lemon. In half (50) of the detection trials they visualized the object whose smell was presented (matched), and in half they visualized a different object (mismatched). As control, 24 subjects received 50 detection trials with no request to imagine anything (no imagery condition). We found that the effect of odor imagery is specific: the difference between matched and mismatched detection was significant for odor, but not for visual imagery. Furthermore, comparisons to the no imagery condition revealed that only the mismatched odor imagery was significantly lower than detection without imagery. We interpret our results as providing further evidence that our paradigm does measure odor imagery. The mechanism of the odor imagery/odor detection effect seems to be interference.

PERCEPTION OF ANIMAL ODORS IN SPACEFLIGHT: SENSORY AND COGNITIVE EFFECTSGould M.¹, Doolittle N.¹, Bateman T.², Dalton P.¹ ¹*Monell Chemical Senses Center, Philadelphia, PA;* ²*BioServe Space Technologies, University of Colorado, Boulder, CO*

Concerns that strong and annoying odors from mouse urine would interfere with astronaut performance precluded mice from flying in manned spacecraft. However, mice offer the scientific community numerous advantages in space science (e.g., larger study populations and transgenic strains). To evaluate the contributions of sensory and cognitive factors on detection and annoyance from mouse odors, we conducted several studies examining the ability of healthy individuals to detect odors from space hardware housing mice. Because prior work identified a type of specific anosmia to selected volatiles from mouse habitats, we evaluated the ability of an electronic nose to reliably detect the presence of urine at concentrations detectable to the 'sensitive' individuals. We also examined the effect of prior knowledge (informative or visual) about the source of the odor on the sensory and hedonic ratings. Consistent with our prior studies, some individuals (10-15%) exhibited a specific, heightened sensitivity to volatiles from the hardware, describing it as moderately strong, urine-like and unpleasant; the majority were unable to detect any odor. Following training, the electronic nose categorized the target odor as mouse urine on 70% of the 'detect' trials. Cognitive factors were also influential in determining the response, with odor ratings increasing over baseline both for individuals that possessed odor source information only and those that visually observed the animals prior to rating. Supported by BioServe Space Technologies, NASA-Ames and NIH DC 03704

MECHANISMS OF OLFACTORY PERCEPTUAL LEARNINGFletcher M.L.¹, Wilson D.A.¹ ¹*Zoology, University of Oklahoma, Norman, OK*

Perceptual learning has been demonstrated in several sensory systems wherein experience enhances sensory acuity for trained stimuli. This perceptual learning is believed to be dependent on changes in sensory cortical receptive fields. Sensory experience and learning have been shown to modify neural response patterns as well as receptive fields in the mammalian olfactory system, however, to date there has been little reported evidence of learned enhancement of behavioral olfactory acuity in rats. The present report used a bradycardial orienting response cross-habituation paradigm that allowed assessment of behavioral discrimination of nearly novel ethyl esters, and then used the same paradigm to examine odorant discrimination after associative olfactory conditioning with similar or dissimilar odorants. The results demonstrate that associative conditioning can enhance olfactory acuity for conditioned odors as well as similar odors, but not for odors dissimilar to the learned odorant. Furthermore, scopolamine (0.5 mg/kg) injected prior to associative conditioning can block the acquisition of this learned enhancement in olfactory acuity. These results could have important implications for mechanisms of olfactory perception and memory, as well as for correlating behavioral olfactory acuity with observed spatial representations of odorant features in the olfactory system. We are currently looking for single-unit correlates of perceptual learning throughout the olfactory system and preliminary data will be presented. Funded by DC03906 to DAW.

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MAPPING THE MULTI-DIMENSIONALITY OF OLFACTORY EXPERIENCE AND MEMORY

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Although humans are limited in their ability to label odorants, when describing olfactory experiences it is common to reference the source of an odor, which evokes the semantics of other sensory modalities. Moreover, one shared feature of all sensory perception is description in terms of conceptual continuums (e.g., intensity). Given the ability of descriptive language to function as a vehicle of association across sensory modalities, the goal of this study was to delineate the emotional, visual, auditory, tactile, and taste connotations of olfactory experience in order to map relations across modalities, and determine whether the resultant semiotic structures are similar across cultures. The extent to which the multidimensionality of odor experience influenced olfactory memory was also examined. 450 subjects (300 American, 150 Japanese) each rated 10 (out of 30 possible) odorants using a semantic differential scale consisting of 50 pairs of polar adjectives. Three factors emerged: hedonics, intensity, and activity. Recognition memory for the odors was evaluated immediately and at one-week delay. Overall, the results suggest that hedonic distinctiveness (positive or negative) of an olfactory representation is an important factor in the memorability of that odor. Although hedonic factors emerged as primary for both cultures, Americans tended to use adjectives describing emotional experience, whereas Japanese participants used adjectives describing the visual aesthetic or external experience of the odor. Supported by Kao Corporation and NIH RO1 DC 03704

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SEX DIFFERENCES IN RECOLLECTIVE EXPERIENCE FOR OLFACTORY AND VERBAL INFORMATION

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We examined recollective experience as a function of sex for olfactory and verbal information. In the first study, men and women studied a set of highly familiar odors with incidental or intentional encoding instructions. In the second study, participants were presented with a number of sentences. At recognition, participants indicated whether their positive response was based on conscious recollection (remembering), a feeling of familiarity (knowing), or guessing. The results indicated that recollection was higher among women than men, and that familiarity-based recognition was equally large across sex for both types of information. The finding that the sex-related experiential difference disappeared when controlling for verbal proficiency suggests that sex-related differences in activating verbal information play an important role for sex differences in recollective experience.

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EVIDENCE FOR LEFT:RIGHT DIFFERENCES IN ODOR DISCRIMINATION, BUT NOT IN SHORT-TERM ODOR MEMORY

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Olfaction differs from most sensory systems in having largely ipsilateral afferent projections that initially bypass the thalamus on their route to the cortex. Data from anatomical, functional imaging, psychophysical, and brain lesion studies suggest that right hemisphere specialization may be present for some forms of higher-order olfactory processing. In this study, we determined, in 130 right-handed and 30 left-handed subjects, whether "short-term" odor memory or odor discrimination differs on the two sides of the nose and, if so whether such differences are influenced by the overall olfactory ability (as measured by the UPSIT), handedness, or sex. Odor discrimination, but not odor memory, was found – in right-handed and left-handed subjects of both sexes -- to be better on the right than the left side of the nose. This phenomenon was not related to the degree of olfactory ability. Women, on average, evidenced better overall odor discrimination performance than men. This study confirms that the right side of the nose is superior to the left in suprathreshold odor discrimination, and provides evidence that short-term odor memory, per se, does not differ between the two sides of the nose. Supported by Grants PO1 DC 00161, RO1 DC 04278, RO1 DC 02974, and RO1 AG 27496 from the National Institutes of Health, Bethesda, MD USA

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ATTENTION TO GUSTATORY AND OLFACTORY FLAVORS

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Attending to a particular tastant increases the ability to detect weak levels of that tastant; Marks and Wheeler (Chemical Senses, 1998, 23, 19-29) reported that the detectability of a gustatory stimulus, either sucrose or citric acid, was greater when subjects attended to that stimulus rather than the other one. The present study examined the role of attention in detecting weak flavor stimuli when these could be either gustatory (sucrose, citric acid) or olfactory (vanillin). Using a forced choice design, on each trial subjects sipped one solution containing the attended, target stimulus and another containing either the non-target stimulus (at a concentration producing the same probability of detection) or water. (To prevent "smelling" the vanillin before "tasting" it, subjects pinched their nose before taking solutions into their mouth.) Results of the first experiment showed that, compared to control, attention improved the detectability of sucrose but not of vanillin. To test the possibility that subjects could detect the vanillin but failed to label it correctly due to its perceptual similarity to sucrose, a second experiment used citric acid as well. Again, attention did not improve the detectability of vanillin, regardless of the alternative stimuli. Indeed, attention to vanillin actually decreased its detectability, suggesting an intrinsic limitation on the capacity to attend selectively to weak olfactory as opposed to gustatory components of flavors. Supported by NIH grant DC00271-16 to LEM.

EFFECTS OF PEPPERMINT ODOR ON INCREASING CLERICAL OFFICE-WORK PERFORMANCE

Grayhem P.¹, Koon J.¹, Whalen A.¹, Barker S.¹, Perkins J.¹, Raudenbush B.¹ ¹*Psychology, Wheeling Jesuit University, Wheeling, WV*

Previous research concerning the administration of an odorant during cognitive and physical tasks has indicated that peppermint odor can enhance task performance. The present study investigated the ability of peppermint odor to augment those tasks involved in clerical office-work, specifically typing performance, memorization ability, and alphabetization ability. In this within subjects design, participants performed the experiment on two separate occasions, once in the presence of peppermint odor and once in an no-odor control condition. Twenty-eight participants' typing performance was assessed using the TypingMaster® computer program, which measured typing duration, gross speed, accuracy, and net speed. Two typing lists of comparable difficulty were used. Memorization ability was measured using an electronic memory apparatus. Alphabetization was measured according to the number of words alphabetized in 30 sec, taking into consideration the number of errors. Two lists of comparable difficulty were used here also. The odor condition and the order of the tasks were randomized. Results indicated a significant difference in the gross speed [$t(24) = -10.269, p < .001$], net speed [$t(24) = -8.76, p < .001$], and accuracy [$t(25) = -2.13, p < .05$] on the typing task, such that peppermint odor improved performance. Alphabetization ability also improved significantly under the odor condition [$t(25) = -3.36, p < .01$]. There were no significant results found for memorization [$t(25) = -.86, p > .05$]. The results of the current study suggest that the presence of peppermint odor may promote a general arousal of attention, thus keeping participants focused on their clerical tasks.

EFFECTS OF ODORANT ADMINISTRATION ON RATINGS OF PHYSICAL ATTRACTIVENESS AND PERSONALITY CHARACTERISTICS

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Physically attractive individuals are more likely to be perceived as possessing positive characteristics, such as intelligence, sociability, and good mental health, than are unattractive individuals (Feldman, 1998). Further, Clark and Waddell (1983) found that when in a positive mood, people are more likely to be attracted to others, and Dutton and Aron (1974) found that the more physiologically aroused people are the more attractive they rate others. This information is useful in conjunction with findings indicating certain odors increase mood (Ludvigson & Rottman, 1989; Raudenbush, Corley, & Eppich, 2001; Raudenbush, Meyer, & Eppich, 2002) and physiological arousal (Hirsch, 1998). The present study investigated the ability of odors to influence ratings of attractiveness, intelligence, healthiness, successfulness, and trustworthiness. Participants ($n=106$, males=38, females=68) rated photographic head shots of six individuals (3 male and 3 female) on the aforementioned characteristics in either a no odor condition, or in ambient odor conditions of cinnamon, lavender, or peppermint. In the presence of both cinnamon and lavender, males rated other males as more attractive, intelligent, healthy, successful, and trustworthy. Males also rated females as more attractive, intelligent, successful, and trustworthy in the presence of both cinnamon and lavender. Few to no differences were found for females respondents for either male or female figures. The implications are salient in regards to affecting how people can be perceived in everyday social interactions. This research was funded by a grant from Psi Chi to N. Corley.

ALTERING THE EXPERIENCE OF CHEMESTHESIS: INSTRUCTIONAL MANIPULATIONS, ATTENTIONAL FOCUS AND PERCEIVED IRRITATION.

Maute C.¹, Dalton P.¹ ¹*Monell Chemical Senses Center, Philadelphia, PA*

The attentional focus or direction that an individual deploys can determine, to a great degree, the content of their subjective experience. Leventhal et al. (1979) have shown that providing subjects with information about the sensory properties of a cold pressor task significantly reduced the perceived pain experience. In this study we investigated the role of attentional focus, through preparatory information, in producing variations in perceived irritation to a volatile compound (VOC) exposure. Four conditions of attentional focus (odor focus, irritation focus, emotion focus and distraction) were compared during a 20-minute exposure to a negatively perceived odorant/irritant mixture. Odor detection thresholds were evaluated pre- and post-exposure, while during exposure subjects rated odor and irritation intensity as well as condition-specific end points. After exposure, subjects made ratings of health symptoms, mood, odor quality, and location of irritation. Before receiving preparatory information, subjects made baseline ratings of odor intensity and irritation. Consistent with predictions from Leventhal et al. (1979), ratings of perceived irritation at the end of exposure, as well as retrospective ratings of irritation, were lowest in the irritation focus condition. Although preliminary, the present study affirms the role of cognitive processes in chemesthetic experience and suggests interventional strategies that may be effective in modifying the subjective experience of sensory irritation in many real-world situations. Supported by NIH DC 03704

MODULATION OF PAIN THRESHOLD, PAIN TOLERANCE, MOOD, WORKLOAD, AND ANXIETY THROUGH ODORANT ADMINISTRATION

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Based on research showing certain odors significantly increase physiological arousal, cognitive performance, and physical performance in humans, as well as increase pain tolerance in rats, the present study was designed to determine if certain odors could increase human participants' ratings of and tolerance to pain. Participants ($n=158$) placed their hand in a cold pressor pain apparatus (3 C water) and were asked to report pain level using a 0-10 scale every 30 sec, to a maximum of 5 min. During testing, participants wore nasal cannulas that administered either unadulterated low-flow (3L/min) oxygen (control condition), peppermint odor plus oxygen, or jasmine odor plus oxygen. Following the pain test, participants completed questionnaires related to mood (POMS; Reddon, Marceau & Holden, 1985), workload (NASA-TLX; Hart & Staveland, 1988), and anxiety (STAI; Spielberger, Gorsuch & Lushere, 1970). The results indicate peppermint odor significantly decreased ratings of pain over time and increased overall pain tolerance. Participants also reported reduced mental, physical, and temporal workload requirements, decreased effort and frustration, and increased performance and vigor in the peppermint condition. Physiologically, odorant administration resulted in an increase in oxygen saturation and blood pressure. Incorporating past research on peppermint odor administration, the present study further indicates the ability of peppermint odor to distract participants from onerous tasks, and produce a psychological sense of greater performance and well-being. This research was funded by a grant from NASA to B. Raudenbush.

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EFFECTS OF MOTIVATION AND COMPETITIVENESS ON PAIN THRESHOLD AND RESPONSE

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Motivation, competitiveness, and gender differences were tested using a cold pressor pain test. Forty-six participants (22 males, 24 females) were placed into one of three groups, designated by informing the participants how long the "average" person is able to withstand the cold pressor pain test. These groups consisted of 1) a control group, where no information was given concerning the average person's performance, 2) a 30-sec group, in which participants were told the average person can withstand 30 sec of testing, and 3) a 4 min-30 sec group, in which participants were told the average person can withstand 4 min and 30 sec of testing. Participants were asked to rate their level of competitiveness on a scale of 1-10, with 1 being not competitive and 10 being highly competitive, as well as complete a questionnaire related to intrinsic vs. extrinsic motivation. The average tolerance of males (135.33 sec) significantly exceeded that of females (116.05). There was no significant association between competitive ratings and pain tolerance. There was no significant difference found between the participant's type of motivation, either intrinsic or extrinsic, and the amount of time that the participant's hand was immersed. Males rated themselves as more competitive, but there was no difference in pain tolerance based on ratings of competitiveness. There was a significant difference in pain tolerance between the 30-sec group (73.87 sec) and the 4 min-30 sec group (179.38 sec). The results indicate a motivational aspect of pain tolerance, related to a priori instructional protocols. This research was funded by a grant from NASA to B. Raudenbush.

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A TEST OF ASSOCIATIVE ODOR LEARNING

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A major theoretical question in olfactory cognition is the extent to which innate versus learned mechanisms control human olfactory hedonic responses. To test the associative learning hypothesis we performed an evaluative conditioning experiment where emotional experience and exposure to a novel odor were manipulated. Participants were randomly divided into 4 groups: (1) positive experience/+conditioning, (2) neutral experience/+conditioning (3) positive experience/-conditioning and (4) neutral experience/-conditioning. The positive experience was playing an entertaining computer game at session 1 and watching funny film clips at session 2. The neutral experience was two sessions of sitting alone in a waiting room with magazines available. +conditioning = novel ambient odor present during the 'experience'; -conditioning = no ambient odor. Baseline hedonic ratings to five odors (1 novel, 4 familiar) were taken from all subjects when they first arrived for the experiment, subsequent ratings were obtained at the end of the two "conditioning" sessions and then at two time delays (1) 48 hrs post baseline (2) 1 week post baseline. Pleasantness was the key variable of interest. Results revealed no differences in baseline ratings to the novel odor, but at both delay tests 1 and 2, subjects in Group 1 rated the novel odor as significantly more pleasant than subjects in any other group. Ratings to the familiar odors did not differ between groups or across time intervals. This finding gives strong support to the notion that hedonic responses to odors are acquired via learned emotional associations. This research was supported by a grant from Oakland.

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MEMORY FOR INDIVIDUAL ODORS IN GOLDEN HAMSTERS: FUNCTIONAL NEUROANATOMY AND ROLE OF PROTEIN SYNTHESIS

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Individual recognition is vital for maintaining the relationships between individuals in many species. Such recognition may be a specially evolved type of learning and memory. In rodents, olfaction plays a key role in individual recognition. We developed a new behavioral model to test individual recognition by odors in a Y maze. In our experiments, two male hamsters interacted with each other and fought during 3 trials (with 3 min inter-trial interval). The loser was tested in Y maze with the winners that beat them after different periods of delay (30 min., & 1, 3, & 7 days). Losers avoided the arm with cues from winners that beat them. They learned to recognize specific individuals within few seconds and remembered this information for at least 7 days. Treatment of the males with anisomycin (150 mg/kg) 20 min before the encounters resulted in no deficit in short-term memory (30 min) but a significant deficit in long-term memory (1, 3 & 7 days). Thus, short-term recognition does not require protein synthesis, but long-term individual recognition does. We also carried out immunohistochemistry to localize c-Fos and Egr-1 expression in males during memory acquisition and retrieval (1-day condition). Our data suggest that specific brain regions (e.g. medial amygdala, hippocampus and parts of olfactory cortex) are involved in learned fear of another individual and memory for that individual. Different brain regions seem to be activated during different phases of learning and memory. Supported by grant number 5R01 MH58001-01A1 from NIMH to R.E. Johnston

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Learning and Cognition

MEMORY CONSOLIDATION IN THE ONE-TRIAL LEARNING OF NIPPLE-SEARCH ODORS IN RABBIT PUPS

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Rabbit pups are only nursed for about 3 min once a day and can learn to associate a novel odorant painted on their mother's ventrum with suckling in one nursing, and even if they obtain no milk. When tested 24 h later on a rabbit fur scented with the odorant used during training, conditioned pups but not naive controls show the typical nipple-search behavior. In a preliminary study, we found full expression of the nipple-search response in pups tested 16 h after conditioning but not in pups tested at 1, 4, or 8 h (Hudson, German J Psychol, 17:267-75, 1993). In a reinvestigation of this phenomenon, we now report that independent groups of pups (n=16/group) tested 30 min, 4 h and 8 h after training had significantly lower nipple-search scores than pups tested 24 h after training (ANOVA). Although naive controls tested at the same time also showed some search behavior their scores were significantly lower at all times than their conditioned siblings. These results indicate that a memory trace for the conditioned odor forms early but confirm that the full expression of the behavioral response is delayed for several hours at least. Currently we are checking whether this delay can be attributed to circadian factors by testing pups 28 h after conditioning. However, this explanation seems unlikely given that rabbit pups will display nipple-search behavior at any time of day. It is hoped that this paradigm will prove useful for investigating the temporal patterning of events underlying the formation and retrieval of olfactory memory. (CONACyT 114697; PAPIIT IN217100)

FLAVOR AND TEXTURE PERCEPTION OF DAIRY PRODUCTS USING PROP CLASSIFICATION AND FREE-CHOICE PROFILING

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The relative importance of flavor versus texture perception of fat is not well understood. Supertasters of PROP perceive more fattiness in salad dressings and fluid dairy products, which could be related to greater trigeminal input. Taken together, these data suggest that Supertasters perceive more textural cues from fat containing foods than Nontasters. The objective of this study was to obtain an in-depth understanding of how flavor and texture are perceived in dairy products using PROP classification of subjects and Free-Choice Profiling (FCP). FCP is a type of descriptive analysis that allows subjects to rate products based upon individually created lists of descriptors. Ten Nontasters and 10 Supertasters were classified via the 1-solution method (Tepper et al., 2001) and rated nine dairy products on idiosyncratic lists of terms using a 15-cm line scale. Generalized Procrustes Analysis captured ~67% of the variance in fat perception (VAR) for both groups in 3 dimensions. Dimension 1 for Supertasters was related to textural and dairy flavor attributes (34% VAR); Dimension 2 (20% VAR) corresponded to the basic tastes of sweet and sour with additional textural attributes. The dimensions were reversed in order for Nontasters, and had equal variance accounted for (28% and 26% VAR, respectively); fewer textural terms were used throughout. Dimension 3 was similar for both groups. These data suggest that Nontasters and Supertasters use different cues to judge fat in dairy products with Supertasters relying more heavily on texture attributes.

POSTINGESTIVE EFFECTS OF ADDED GLUTAMATE ON LIKING FOR NOVEL FLAVORS

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Adding glutamate to suitable foods increases their umami quality, and their acceptability and intake, even in human neonates. The functional significance of this universal palatability is unclear. Other highly palatable substances, e.g., sugar and fats, increase liking for novel flavors with which they are repeatedly paired, especially when ingested. This is thought to reflect the rewarding effects of providing energy. To determine if umami palatability might also reflect the use of glutamate as energy, 44 subjects (Ss) rated 10 ml samples of three novel soups for liking and familiarity before and after seven daily exposures to each of two of these soup flavors – one with added MSG (0.5% w/w; MSG+) and one without (MSG-). During exposure, Ss received either a 250 ml bowl of soup (Consume group) or a 10 ml sample (Taste group). The Consume group had a greater increase in liking for the MSG+ flavor than for either the MSG- flavor or non-exposed control. There were no significant sample differences in the Taste group. For the MSG+ sample, there was a greater increase in liking in the Consume group than in the Taste group. In Exp. 2, 69 Ss were divided into three groups (Consume MSG+; Consume MSG-; Taste MSG+) in which they were exposed to only one novel soup flavour. The Consume MSG+ group showed a greater increase in liking than either the Consume MSG- or the Taste MSG+ groups, which did not differ. Changes in familiarity ratings reflected amount consumed, not MSG content. Thus, pairing a novel flavor with the post-ingestive effects of added glutamate can condition liking. This suggests that, as with carbohydrates and fats, post-ingestional absorption of glutamate is rewarding, and may signal its use as an energy source.

EFFECTS OF CAPSAICIN TREATMENT ON TASTE PERCEPTION IN WOMEN

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Our objective was to see if capsaicin-induced oral burning would produce taste-intensity decreases similar to those seen in a BMS population (Formaker and Frank, 2000). Thirteen healthy women (mean age (SD), 24.3 (2.4) yrs) participated in two taste sessions. Within each taste session, stimuli were presented randomly over two replicates. The stimuli were five concentrations (in half-log steps) of NaCl (salty, 0.01-1.0M), sucrose (sweet, 0.01-1.0M), citric acid (sour, 0.32-32mM) and quinine-HCl (QHCl, bitter, 0.01-1.0mM). Stimuli were rated for intensity and their quality identified. A subject was treated (once before and again halfway through each replicate) with 10ppm capsaicin in one session and water in the other session. The data showed that intensity ratings for the induced burn decreased by 83% as a function of the cumulative number of judgments. Water control rinses never induced oral burning. Capsaicin treatment resulted in significant overall reductions in taste intensity ratings for citric acid (22%) and QHCl (27%) relative to the water control rinse. NaCl showed a trend toward decreased intensity at the highest concentrations. Sucrose was not affected by the capsaicin rinse. The current study demonstrated that decreases in taste intensity occurred with temporary trigeminal nerve activation by capsaicin. However, capsaicin affected intensity ratings for different stimuli than BMS and, in contrast to BMS, capsaicin did not affect the identification of detected stimuli. This suggests that the presence of a burn alone does not account for the effects of BMS on taste perception. This study was supported by UCHC.

HORMONAL GATING OF EXPOSURE-INDUCED SENSITIVITY TO ODORS IN WOMEN

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Induction of olfactory sensitivity in humans was first illustrated following exposure to the volatile steroid androstenone (5 alpha-androst-16-en-3-one), when men and women who were initially unable to smell this compound developed that ability following repeated, brief exposures. Recently we showed that induction of enhanced sensitivity is a more general phenomenon, with dramatic changes in olfactory acuity occurring during repeated test exposures to several odorants among women of reproductive age, but not men. To investigate the possibility that female hormones enabled this sensitization, we evaluated threshold changes following repeated threshold test sessions for eighteen female subjects (48-75 yo) who had been post-menopausal for at least two years. Sensitivity changes in 9 subjects on hormone replacement therapy (HRT) were compared with changes in control subjects, matched for age and time since menopause, who had never used HRT. Two odor thresholds for benzaldehyde were obtained at each of 8 sessions; to evaluate the specificity of any changes, thresholds for a control odorant, citralva, were obtained before and after these sessions. Saliva samples to verify estradiol levels were collected at the beginning and the end of the test. There was a significant interaction between group and time ($p < 0.02$), with women using HRT showing significantly greater sensitization to benzaldehyde than women not on HRT. No comparable changes were observed for the control odor among either group, confirming the relative specificity of the enhanced sensitivity. Supported by NIH RO1 DC 03704 and DC 02995.

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OLFACTORY CODING OF PLEASANTNESS AND INTENSITY IN THE HUMAN AMYGDALOID COMPLEXSobel N.¹, Khan R.¹, Stappen I.¹, Anderson A.¹ ¹*Neuroscience, University of California, Berkeley, CA*

Imaging has shown amygdaloid complex (AC) activation in response to aversive odors. Odor quality is dependent on odor intensity and pleasantness. Here we dissociate these dimensions to ask whether AC activity reflects odor pleasantness, intensity, or both. An olfactometer generated 5 stimuli; 1) PH - pleasant high intensity (5.66 mol/l citral), 2) PL - pleasant low intensity (0.11 mol/l citral), 3) UH - unpleasant high intensity (0.979 mol/l valeric acid), 4) UL - unpleasant low intensity (0.041 mol/l valeric acid), 5) clean air. 16 subjects participated in an event-related fMRI study at 3T (T2* spiral, TE= 30, TR = 1s, 64x64 FOV, 17 slices, thickness = 4mm, stimulus ISI = 20s, stimulus repetition = 30). First-pass analysis (SPM99) to compare for regions of increased activity related to odor intensity regardless of pleasantness revealed a pronounced locus of activity in the AC ($P < .001$). In contrast, an analysis comparing for increased activity related to unpleasantness regardless of intensity, revealed a locus of activity in the orbitofrontal gyri ($P < .001$). In-depth ROI analyses corroborated these findings, showing activity in the AC was significantly correlated with individual intensity rating ($r = .35$, $p = .001$) but not with pleasantness rating ($r = .04$, $p = .7$). These findings suggest that the AC may be encoding more of the immediate physical dimensions of odor (intensity) rather than the later "psychological dimensions" (pleasantness). Funding: Searle, NIH-NIDCD, SORI

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CEREBRAL PROCESSING OF BIMODAL ODORANTSBerglund H.¹, Heden-Blomqvist E.², Savic I.³ ¹*Medicine, Karolinska Institute, Stockholm, Sweden*; ²*ENT, Karolinska Institute, Stockholm, Sweden*; ³*Neuroscience, Karolinska Institute, Stockholm, Sweden*

Cerebral regions mediating the separate components of bimodal odorants are rather unknown. The pattern of cerebral activation was therefore investigated during odorous stimuli in 12 healthy controls and 12 patients with peripheral anosmia. Methods: [15O]-H₂O-PET scans were conducted during passive smelling of the unimodal odorant vanillin (VAN), a bimodal odorant acetone (ACE), and odorless air (AIR). Activations were calculated contrasting VAN and ACE to AIR, and deactivations, running these contrasts in the opposite direction. Results: Controls: VAN activated both sides amygdala and piriform cortex. ACE activated only a minor portion of these regions, exclusively on the right side. Instead, clusters were found in anterior insula, cingulate and somatosensory cortex, cerebellum, thalamus, hypothalamus, and medulla. In parallel, the secondary somatosensory, visual and auditory cortices were deactivated. No deactivations were observed with VAN. Anosmics: No engagement of olfactory regions was observed with VAN. During ACE these regions were even deactivated. The pattern of activation in ACE was otherwise similar to controls. Summary and conclusion: 1) The olfactory cerebral circuits processing unimodal odorants seem to be only partly engaged by bimodal odorants. 2) Processing of bimodal odorants strongly engages trigeminal cerebral projections, with a similar distribution in anosmics and controls. 3) Activation with bimodal odorants is not a sum of its components. Rather, the olfactory areas seem to be inhibited by bimodal odorants' trigeminal component. This deactivation comprises, however, also other modalities and can be attributed to attentional shift.

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OLFACTORY IMPAIRMENT IN A POPULATION AT RISK FOR DEMENTIAMurphy C.¹, Schubert C.², Cruickshanks K.J.² ¹*Psychology, San Diego State University, San Diego, CA*; ²*Ophthalmology and Visual Sciences, University of Wisconsin-Madison Medical School, Madison, WI*

As new clinical trials for Alzheimer's drugs are conducted and treatments become available, the need for tests that identify persons at high risk for the disease is increasingly urgent. One group of elderly persons at risk for Alzheimer's disease are those with cognitive impairment. Since the earliest site of development of neurofibrillary tangles in Alzheimer's disease is the entorhinal cortex, an area involved in processing olfactory information, olfactory tests may prove to be useful in early diagnosis. We sought to investigate whether persons from an epidemiological sample of community-dwelling elderly with cognitive impairment would show olfactory impairment on a rapidly-administered 8-item odor identification test. To directly address this issue, we examined the olfactory function of 2,488 community-dwelling elderly persons, participants in a population-based epidemiological study, who ranged from 50-97 years of age. Of these, 161 had either an established diagnosis of Alzheimer's disease or a score on a mental status test that would qualify them as cognitively impaired. All were individually administered the San Diego Odor Identification Test, an 8-item test which uses natural common odors. The items were presented one at a time while the subject closed the eyes to prevent visual cues. A picture board with illustrations of these items as well as distractors was presented to aid in identification. The results suggest that a rapidly-administered odor identification test can be useful in identifying persons with cognitive impairment in the general population. Such a test may be useful in identifying candidates for primary prevention of dementia. Supported by NIH grants AG11099 and AG04085 from the National Institute on Aging.

128 Symposium Behavioral Analysis of Chemosensory Function

INTRODUCTION: BEHAVIORAL ANALYSIS OF CHEMOSENSORY FUNCTIONGlendinning J.I.¹ ¹*Barnard College, Columbia University, New York, NY*

This symposium will highlight the value of behavior as a tool for studying chemosensory function. All four of the speakers work with different model systems, and have extensive experience using behavioral tasks to study sensory function. Robert Dooling will review the behavioral paradigms for measuring auditory perception in birds. He will show how these paradigms have provided deep insight into how birds recognize, discriminate, and classify naturally occurring vocalizations. Brian Smith will discuss olfactory coding mechanisms in insects. He will illustrate how molecular, anatomical and physiological features of the olfactory system establish the basic elements of the code, and then go on to show how experience with odors can modify this code. Matthias Laska will examine olfactory function in mammals. He will discuss how behavioral tests have become an essential tool for evaluating many of the hypotheses generated by molecular, genetic, and cellular studies of the olfactory system. Finally, John Glendinning will review the behavioral paradigms for studying gustatory function in mammals. He will underline the risks associated with becoming over-reliant on a single behavioral testing procedure (i.e., two-bottle preference tests), and emphasize the importance of developing a variety of taste-related behavioral tasks.

129 Symposium **Behavioral Analysis of Chemosensory Function**

AVIAN PSYCHOACOUSTICS

Dooling R.J.¹ ¹*Psychology, University of Maryland College Park, College Park, MD*

There have been tremendous advances in the methodology of comparative psychoacoustics over the past 25 years and advances in computer-aided experimental control and in digital signal synthesis and analysis are primarily responsible. To be effective, psychoacoustic paradigms must also be tailored to the specific behavioral adaptations of different animal species. Examples of specific paradigms for measuring auditory perception in birds are described. Some of these procedures, using simple sounds such as pure tones and noises, provide an assessment of basic auditory function such as the audiogram with a degree of experimental rigor that now rivals that typically found in human psychophysical studies. For complex sounds such as human speech and animal vocalizations, variations of these procedures are now able to measure more complicated aspects of perception such as the discrimination, recognition, and classification of naturally-occurring vocalizations and the existence of perceptual categories. These procedures require a reliable testing procedure, a response metric such as reaction time which shows a high correlation with stimulus similarity, and sophisticated statistical procedures such as cluster analysis and multidimensional scaling. Together these techniques provide a way of assessing the extent to which the world sounds different to humans and animals.

130 Symposium **Behavioral Analysis of Chemosensory Function**

EVOLUTION IN PARALLEL: WHAT BEHAVIORAL STUDIES OF INSECTS TELL US ABOUT OLFACTORY CODES IN GENERAL

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The phylogenetic relationship among invertebrate and vertebrate olfactory systems remains an enigma. Physiological and anatomical features of olfactory coding are very similar, yet molecular features are very different. Further comparative analysis might yet reveal a convergent means for coding a very large number of odors. Many insects must have the capacity to detect a large number of odors because odors often change meaning several times within an animal's lifetime. Indeed many insects can learn to discriminate a very large number of odors. In order to understand how such a broad-based olfactory code is manifested in behavior we have to consider how molecular, anatomical and physiological features set up basic elements of that code. Behavioral studies of stimulus generalization in insects have shown that odor concentration and molecular features such as, carbon chain length, shape, and functional group regulate perceptual features of an odor. But these features are insufficient for a complete characterization of odor coding because the nature of experience with an odor also influences the perception of that odor. Certain types of experience – lack of reinforcement and contextual cues – can alter the shape of a generalization gradient. And the specific type of experience with a blend can influence how that blend is perceived. In conclusion, behavioral studies of olfactory coding in insects have revealed many features that parallel those found in vertebrates. Future, more detailed analyses stand to reveal the relationship of this general problem to specific communication by way of pheromones, allomones and kairomones. This work was supported by NIH-NCRR (9 R01 RR14166).

131 Symposium **Behavioral Analysis of Chemosensory Function**

WHAT GENES, SECOND MESSENGERS, AND ION CHANNELS CANNOT TELL US.....OR: WHY BEHAVIORAL ANALYSIS OF OLFACTORY FUNCTION IS MORE IMPORTANT THAN EVER

Laska M.¹ ¹*Dep. of Medical Psychology, University of Munich, Munich, Germany*

Enormous progress has been made in recent years with regard to our understanding of chemosensory function at the genetic and the molecular level. However, these new findings, for example concerning the repertoire of genes coding for olfactory receptors or the mechanisms underlying the olfactory primary process raise new questions which can best, if not exclusively, be addressed at the behavioral level. Thus, in addition to their more obvious and traditional role in studying the biological significance of intra- and interspecific chemical communication, cognitive functions such as odor learning and memory, and the capabilities and limits of a species' olfactory system in general, behavioral assays gain new importance as they also allow us to test hypotheses generated by findings that employed genetic or molecular approaches. Odor structure-activity relationships, arguably one of the central topics in olfactory research, are a good example: The finding that the mammalian genome is coding for approximately 1,000 different types of olfactory receptors, allows a re-evaluation of discrimination performance with structurally related substances as determined at the behavioral level. The emergence of genetically modified animals such as knock-out strains of rodents is another example of the re-newed importance of behavioral assays as such animal models provide an excellent new means of studying the impact of genetic factors on olfactory function. To this end, appropriate behavioral assays need to be developed – a challenge for behavioral biologists.

132 Symposium **Behavioral Analysis of Chemosensory Function**

WHAT IS THE BEST STRATEGY FOR ASSESSING TASTE-MEDIATED INGESTIVE RESPONSES IN MICE?

Glendinning J.I.¹ ¹*Department of Biological Sciences, Barnard College, Columbia University, New York, NY*

Our understanding of how taste stimuli are transduced and processed along the gustatory neuraxis of mice has improved dramatically over the last 5 years. This progress has increased the demand for behavioral tests that can (a) identify animals with taste dysfunction quickly and reliably, and (b) pinpoint the nature of the dysfunction. Most studies of taste function in mice, however, employ a single behavioral testing procedure: two-bottle preference testing. I will argue that the reliance on a single testing procedure, particularly one that is low throughput and frequently confounded by non-gustatory factors, is an ineffective strategy for meeting the goals outlined above. Instead, investigators would increase their chances of success by employing several complementary testing procedures. For example, there are testing procedures that measure the hedonic aspects of taste (e.g., the ability of taste stimuli to stimulate or inhibit feeding and drinking) and others that measure the sensory/discriminative aspects of taste (e.g., detection thresholds or ability to discriminate taste stimuli). I will discuss examples in which the exclusive use of a single testing procedure has produced misleading results. Further, I will describe a new high-throughput and automated testing procedure that my colleagues and I have developed, which should complement the results obtained from two-bottle preference testing.

SWEDISH SMELL IDENTIFICATION TEST FOR CHILDREN (SSIT-C)

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Little is known about the development of olfactory functions in children. The Swedish Smell Identification Test for Children (SSIT-C) is developed to address a culturally valid odor identification test for clinical use in the Swedish population. In the current data-collection, healthy children ranging in age from 5-14 years are assessed in olfactory sensitivity (i.e., absolute threshold for Butanol) and odor identification. The identification test includes 16 common odorants (e.g. chocolate, banana, lemon) and performance is assessed both by means of free identification, and by a more supportive multiple-choice procedure. In instances when the child fails to spontaneously name the odor, three different pictorial response alternatives are provided (one target and two foils). This procedure was chosen because of the well-documented difficulties in naming odorants and that failures in free identification not necessarily imply that the subject lack knowledge of the odorant's name. Preliminary results suggest an age invariance in olfactory sensitivity, and a gradual increment across age in identification performance for both types of retrieval formats. No sex differences were observed in any of the olfactory tasks.

ASSESSMENT OF OLFACTORY FUNCTION IN CHILDREN USING THE SNIFF MAGNITUDE TEST

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A new measure of olfactory function, the Sniff Magnitude Test (SMT), has recently been found to be valid and reliable when testing younger and older adults. Assessment of the sense of smell is difficult in children because of limited attention spans, undeveloped language abilities, and lack of familiarity with odors and names. Because the SMT minimizes the impact of these factors, it may be a useful method when testing children. The device is unique because the dependent variable does not rely on a verbal response, which has proven to be problematic when testing children. The present study assessed olfactory function in children compared with younger adults. Children aged 3 to 9 years were tested using both the SMT and the University of Pennsylvania Smell Identification Test (UPSIT). Negative air pressure changes associated with sniffing methylthiobutyrate were quantified using a piezoelectric pressure transducer which produced output that was digitized, stored, and analyzed to yield measures of sniff duration and magnitude. Previous work in our laboratory indicated that normosmics reduce their sniff magnitude and duration in response to the malodor, while anosmics do not. Preliminary results showed that when compared with younger adults, children scored significantly lower on the UPSIT. However, there were no significant differences between children and adults when assessed with the SMT. Results are consonant with previous research and suggest that the SMT can be useful in the assessment of children. Supported by NIH DC-4139-01A1 to RCG

BITTER TASTE MODIFICATION BY SODIUM SALTS IN PEDIATRIC POPULATIONS AND ADULTS

Pepino M.Y.¹, McFarlane N.¹, Teel T.¹, Breslin P.A.¹, Beauchamp G.K.¹, Mennella J.A.¹ ¹*Monell Chemical Senses Center, Philadelphia, PA*

The palatability of oral medications, many of which are quite bitter, plays an important role in achieving compliance in pediatric patients. The present study aimed to test the hypothesis that for children and like adults, the addition of sodium salts to some, but not all, bitter tasting liquids enhances acceptance and reduces the perceived bitterness. Using methodologies that are sensitive to the cognitive and behavioral limitations of children, we examined the sensory and hedonic responses of 7- to 10-year-old children and their mothers individually. Following training, subjects were presented with pairs of solutions that differed in the presence of a variety of bitter stimuli and a sodium salt. Each was asked to indicate which of the pair tasted more bitter during one test session and which of the pair tasted better during another. Solutions were also ranked from most to least preferred. For both children and adults, salt significantly suppressed the perceived bitterness and enhanced the acceptance of urea and caffeine, whereas the reverse was true for another bitter stimulus, Tetralone.TM Taken together with the finding that children in the present study preferred the salted solutions more than adults, these data suggest that the use of salts in suppressing the bitterness of some bitter agents may be an especially effective strategy for pediatric populations. This research was supported by NIH Grant HD37119.

TASTE PORE DENSITY ON THE TONGUE AND PROP SENSITIVITY IN CHILDREN

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The aim of the study was to correlate taste pore and fungiform papillae densities on the tip of the tongue with a psychophysical measure of PROP sensitivity in children. Forty 8-9 year old children (20 female, 20 male) were studied. At the first session each subject was trained to use a 9 point category scale and then they rated the strengths of NaCl, sucrose and PROP solutions. At a second session a small (3mm x 3mm) region on the anterior tongue was stained with methylene blue and examined at high magnification (X180). Videomicroscopy recordings were examined using NIH Image software and the number of taste pores were counted on each fungiform papilla. The results showed that there was a significant correlation between taste pore density and perceived intensity for each of the concentrations of PROP for both male and female children. The density of fungiform papillae was also significantly correlated to the perceived intensities of each level of PROP. Individuals were assigned to PROP taster groups and there was a greater proportion of female supertasters. Supertasters rated the high concentration of sucrose significantly stronger than the nontasters did. The results of this study show that 8-9 year old children vary in their taste function and anatomy in a similar way to adults. It is now feasible to study children's food preferences and behaviors in relation to taste function and taste anatomy on the tongue.

GENETIC SENSITIVITY TO 6-N-PROPYLTHIOURACIL (PROP) INFLUENCES ACCEPTANCE OF CERTAIN BITTER AND SPICY FOODS IN PRESCHOOL CHILDREN

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Background: Previous studies in young children from this laboratory found an association between the inherited ability to taste 6-n-propylthiouracil (PROP) and lower acceptance of bitter and high-fat foods. Objective: In the present study, we follow-up and expand upon previously reported findings in a different group of children. Methods: Fifty-three, 4-5 y old children (27 boys, 26 girls) were classified as tasters (T) (N=34) or nontasters (NT) (N=19) using a standard screening solution. Children rated acceptance of common bitter and fat-containing foods with a 5-point facial scale. Anthropometric measures were taken in the laboratory. Mothers completed questionnaires to assess children's preferences for an additional 59 foods and also listed their child's most and least liked foods. Results: (T) gave lower ratings to grapefruit-orange juice in the laboratory ($p < 0.05$). On preference questionnaires, (T) reported lower ratings for spicy mustard ($p < 0.05$) than (NT). In addition, there were non-significant trends for (T) to report lower scores for hot sauce and tabasco sauce ($p < 0.10$). Spicy foods were listed as some of the least liked foods for (T), but were not mentioned for (NT). Multiple linear regression models to predict the reported liking of foods on the questionnaire were performed, and the model for grapefruit juice was significant, with maternal PROP status predicting 28% of the variance ($p < 0.05$). Conclusion: These data provide further support that PROP status influences food acceptance patterns in children and suggests that this association might be stronger for foods with more intense taste qualities.

THE EFFECTS OF TIMING AND DURATION OF EXPOSURE ON ESTABLISHING FLAVOR PREFERENCES DURING INFANCY

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The study of the infants' acceptance of formulas containing protein hydrolysates provides a model system for evaluating the role of early human sensory experiences on later preferences. To most adults, the flavor of these formulas is judged highly unpalatable, having unpleasant taste and olfactory characteristics. When first introduced, infants younger than four months of age will readily accept and consume these formulas, whereas older aged infants, like adults, will strongly reject them. However, if an infant is exposed to these formulas during this early period of acceptability, the formula remains acceptable for a considerable period of time thereafter. The goal of the present longitudinal study was to determine the period during early infancy when exposure to protein formula renders it acceptable to 7- to 8-month-old infants. To this end, infants, whose parents had chosen to formula feed them, were randomized into one of the four groups by the second week of life. One group of infants was assigned to be fed a milk-based formula (Enfamil) whereas another was assigned to be fed a protein hydrolysate formula (Nutramigen) during the entire 7-month period of the study. The remaining two groups were assigned to feed Nutramigen for three months and Enfamil for four months; the timing of exposure differed between the groups. After this 7-month exposure period, infants were videotaped while feeding Enfamil on one test day and Nutramigen on another. Preliminary analyses revealed that acceptance of hydrolysate formulas was significantly enhanced among those infants who received the longest and/or most recent exposure. Supported by NIH Grant HD37119 and a grant from The Annenberg Foundation.

HEIGHTENED SOUR PREFERENCES DURING CHILDHOOD

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Despite the striking paucity of basic research on preferences for sour taste, industry has clearly found a strong niche for 'extreme' sour candies in children. The present study aimed to determine the level of sourness preferred in a food matrix in children and adults. To this aim, preference for sourness in lemon-flavored gelatin was determined by individually testing 61, 5- to 9-year-old children and their mothers. Subjects were asked to rank from most to least preferred four gelatins that differed in citric acid content. A ranking of the gelatins from most to least sour was performed on a separate test day. Mothers completed a variety of questionnaires and children were asked several questions to assess whether children's temperament and food preferences and habits contribute to sour preferences. Although each child and adult was able to rank the gelatins from most to least sour in an errorless fashion, children preferred significantly higher levels of sourness when compared to adults. More than one third of the children, but virtually none of the adults, preferred the most sour gelatins. These children were significantly more likely to prefer extreme sour flavors in other foods and were significantly less neophobic when compared to the remaining children. Whether preferences for extreme sour tastes in children are due to generalized preferences resulting from repeated exposures to sour flavors, individual/ontogenic differences that underlie sour perception, personality factors or a combination of the above remains to be determined. Supported by NIH Grant HD37119.

INFLUENCES ON YOUNG CHILDREN'S WILLINGNESS TO TRY NOVEL FOOD

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Will social cues by a peer or parent influence a young child's willingness to try new foods? In this experiment young children watched a video of a mother and son trying a new food and responding by either no emotion, like or dislike. The subjects were 20 male and female 3- to 6-year olds who attend the Tufts Educational Day Care Center. The methodology consisted of three parts: two training segments and the experimental trials. Subjects were trained to use a five-point Likert scale. In trial 1, a non-emotional scene was shown with the mother and peer trying a common food in an uncommon combination. Trial 2 and 3 used somewhat common foods that are commercially available in unusual variations. Trial 4 used a laboratory concocted food that would be novel to all subjects. The order of food presentation was kept consistent for all subjects and the presentation of emotional responses was counterbalanced. With no emotional information, subjects refused to try the novel food on the first request 40% of the time. Given emotional information, subjects refusal decreased to 15-25% on the first request to try the three novel foods. The results suggest some emotional response is necessary for young children to try foods at all. Additionally, subjects' rated the novel food's taste as more positive if both models said that food is yummy.

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STRUCTURAL AND FUNCTIONAL MEASURES OF MATURATION IN CULTURED HUMAN OLFACTORY NEURONSGomez G.¹, Rawson N.¹ ¹Monell Chemical Senses Center, Philadelphia, PA

Mature olfactory neurons *in vivo* are identified by morphology, molecular markers, and odorant sensitivity; their ability to regenerate throughout adulthood makes them an ideal *in vitro* model for olfactory neurogenesis. In identifying mature neurons *in vitro*, however, there appear to be dichotomous perspectives on the precise criteria of maturity: most studies have emphasized the occurrence of specific molecular markers that are believed to signal maturation *in vivo*, while a few have also considered the ability of cells to respond to odorant stimulation. No studies have correlated the presence of specific molecular markers with the development of odorant responsiveness. To develop a definition of maturation that encompasses both functional and anatomical characteristics, we focused our studies on evaluating the expression of specific protein markers in functionally characterized odorant responsive neurons. Cells were grown *in vitro* using cell culture techniques. On each day from one to six days after a passage, a subset of cells was tested for their odorant sensitivity and subsequently tested for OMP and NCAM immunostaining. We quantified the degree of co-occurrence of marker molecule expression with the acquisition of odorant sensitivity. We found that while all odorant responsive neurons were NCAM+, some functionally mature cells were OMP-, indicating that OMP is not necessary for functional maturation *in vitro*. Additional marker molecules are being tested. These results have important implications for the evaluation of cell differentiation using *in vitro* systems, and provide a basis for developing a better understanding of the process by which olfactory neuron maturation occurs *in vitro*. Supported by NIH DC00214.

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NT-3 INCREASES THE SURVIVAL OF MATURE ORNS AND REDUCES THE PROLIFERATION AND MATURATION OF IMMATURE NEURONSWang E.¹, Simpson P.J.¹, Moon C.¹, Samanta-Roy D.¹, Ronnett G.V.¹ ¹Neuroscience (SOM), Johns Hopkins University, Baltimore, MD

Neurotrophins have profound affects on various aspects of neurogenesis. Our goal was to characterize the role of the neurotrophin NT-3 in the olfactory system. Mice with targeted deletions of NT-3 demonstrate increased apoptosis in mature neurons within the olfactory epithelium, accompanied by decreased apoptosis of immature neurons and increased proliferation of neuronal precursors. An *in vitro* dissociated cell culture system was used to study the mechanism of these effects. *In vitro* NT-3 treatment increases the number of mature neurons and reduces the proliferation of neuronal precursors. The effect of NT-3 on the survival of mature neurons is prevented by the addition of a NT-3 activity-blocking antibody. In contrast, NT-3 blocking antibody does not affect the proliferation or maturation of immature NST positive neurons as shown by process outgrowth, suggesting that secondary factors are responsible for these effects. Analysis of the signal transduction pathways was also performed and it was shown that at least two different pathways are involved in mediating these effects. These results suggest that NT-3 plays an important role in regulating maturation and survival of ORNs by both direct and indirect mechanisms.

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SPATIALLY DYNAMIC EXPRESSION OF MECP2 IN RODENTSCohen D.¹, Matarazzo V.¹, Palmer A.¹, Khokhar B.¹, Ronnett G.V.¹ ¹Neuroscience, Johns Hopkins University, Baltimore, MD

MeCP2 is a transcriptional repressor that specifically recognizes DNA methylated at cytosine residues. Recently, mutations in this protein have been shown to be responsible for Rett Syndrome (RTT), a pervasive neurodevelopmental disorder. This disease is characterized by the development of seizures, autism, and motor dysfunction primarily in girls between the ages of 6-18 months. Olfactory biopsies from Rett patients have revealed morphological abnormalities in olfactory receptor neurons (ORNs) in the olfactory epithelium. We have used the olfactory system as a model in which to investigate the developmental role of MeCP2. We have characterized the developmental time course of MeCP2 expression immunohistochemically. We find expression of MeCP2 in both olfactory epithelium (OE) and olfactory bulb (OB), although expression is consistently higher at all ages in the olfactory bulb. In the OE, MeCP2 expression is highest in sustentacular cells, and is variable in cells of the olfactory receptor neuronal lineage. The variability in neuronal staining varies throughout the epithelium, depending upon location along the dorsal/ventral axis. To further understand MeCP2 function, we have performed detergent ablation of the epithelium and unilateral olfactory bulbectomies. In each case, we see strong MeCP2 expression in cells undergoing mitosis, suggesting that MeCP2 may be involved in the regulation of proliferation. In addition, MeCP2 is expressed in immature neurons, indicating that MeCP2 may function in the maturation of neuronal precursors. Together, these results suggest that MeCP2 expression is dynamic and appears to be involved in the regulation of gene expression during development of ORNs. Supported by IRSA and NIH

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EXPRESSION OF DLX 1 AND 2 IN THE NEONATAL AND ADULT MOUSE OLFACTORY SYSTEM.Saino S.¹, Baker H.¹ ¹Neuroscience, Weill Med. Coll. Cornell, Burke Med. Res. Inst., White Plains, NY

Dramatic reductions in the number of olfactory bulb (OB) granule and periglomerular cells in neonatal *Dlx 1/2* null mice suggest that these interneurons originate from *Dlx 1* and *2* expressing progenitors of the lateral ganglionic eminence. In adults, new precursors are generated from stem cells in the anterior subventricular zone (SVZa) that migrate in the rostral migratory stream (RMS) to the OB. To begin defining the role of *Dlx 1* and *2* in the generation, migration and differentiation of postnatal and adult OB interneurons, this study examined the distribution of the *2* genes and their protein products in the neonatal and adult subventricular zone, RMS, OB as well as other brain regions using non radioactive *in situ* hybridization and immunocytochemistry. In adults, discontinuous, strong expression of *Dlx 1* and *2* mRNAs and *Dlx 2* protein was found in the lateral and anterior walls of the lateral ventricles. The heavily labeled RMS followed a serpentine path that continued into the OB where both granule and periglomerular cells expressed *Dlx 1/2*. In the ependyma adjacent to the alveus of the hippocampus, label was continuous with that in the ventricular zone. Cells were found in many forebrain regions including the cortex, septum and anterior olfactory nucleus, but not in the mid- and hindbrain. Neonates showed a similar, but more extensive, regional labeling. The staining intensity for both *Dlx 2* mRNA and protein decreased with the RMS > granule cells > periglomerular cells. These findings suggest that *Dlx 1/2* expression is associated with generation of OB interneurons throughout life. Supported by AG09686.

PROGENITOR CELLS AND CONTINUAL DEVELOPMENT OF THE LOBSTER'S OLFACTORY ORGAN

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Olfactory receptor neurons (ORNs) of spiny lobsters (*Panulirus argus*) undergo continual turnover, with cells added in a 'proliferation zone' (PZ) at the proximal end of the olfactory organ and lost from the distal end. Thus a spatio-temporal developmental axis exists in the olfactory organ. We examined the sequence of mitotic events related to formation of olfactory tissue, using the cell proliferation marker BrdU. Proliferating cells first appear as patches in the epithelium, which are located immediately proximal to the PZ. Cells in these patches do not have ORN morphology. Within the PZ (defined as the zone in which ORNs appear), proliferating cells occur first as bi-layered clusters located just above the epithelium. Cells are added to these clusters by localized proliferation and not recruitment. The majority of cells in these clusters have ORN morphology, but the clusters have fewer cells than mature ORN clusters and they are not organized as rows as are mature clusters. Proliferation continues in these clusters until they are fully formed. New clusters form proximal to existing clusters and push these existing clusters vertically away from the epithelium. We believe that the epithelial patches are progenitor cells, which divide, differentiate, and organize into clusters of ORNs and supporting cells. We present a hypothetical model of cellular and molecular events involved in this development sequence. (Supported by NIH DC00312 and NSF IBN-0077474)

HOMOLOGUES OF THE DEVELOPMENTAL GENES HAIRY/DEADPAN AND CYP4 CYTOCHROME P450 IN THE OLFACTORY ORGAN OF SPINY LOBSTER

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The olfactory organs of many animals, including the spiny lobster *Panulirus argus*, show continuous post-embryonic neurogenesis (Harrison et al 2001 J Neurobiol 47:51). Toward our aim of characterizing molecular mechanisms of olfactory neurogenesis, we have used degenerate RT-PCR to identify in the antennule of spiny lobsters homologues of developmental genes. We have identified 2 such homologues, *deadpan/hairy* and *CYP4*. *Deadpan* and *hairy* belong to a family of genes encoding bHLH transcription factors that assist in determining cell fate, by singling out neuronal progenitors within groups of equivalent cells by acting as transcriptional repressors of *achaete-scute* complex genes through Notch (Artavanis-Tsakonas et al 1999 Science 284:770). *CYP4* is a member of a family of cytochrome P450s that are involved in the metabolism of steroids in arthropods and may function in neural development through regulating levels of steroids (Chantal et al 1999 Biochem Biophys Res Commun 264:413; Brennan et al 1998 Development 125:2653). We analyzed the expression pattern of these 2 genes in *P. argus* using RT-PCR and Dot blot. Of 12 tissues examined, expression was limited to sensilla-bearing tissues (antennular lateral and medial flagella, leg tips, carapace), eye (*hairy* only), and brain. We are currently performing in-situ hybridization to examine the cellular expression pattern of these genes. (Supported by NIH DC00312 and NSF IBN-0077474)

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES AT A SITE OF OLFACTORY NEUROGENESIS

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Olfactory receptor neurons (ORNs) are continually replaced in most animals. In the olfactory organ (lateral flagellum of the antennule) of the spiny lobster *Panulirus argus*, ORNs and associated supporting cells normally are generated in a proximally-located proliferation zone and die in the distal portion of the organ. This regional separation of the various processes of cell turnover in the olfactory organ creates a proximo-distal axis of neuronal age, maturity, and function (Steullet et al 2000 J Neurosci. 20:3282, Harrison et al 2001 J Neurobiol 47:51). Taking advantage of the anatomical separation of mature ORNs from their progenitors, we used a PCR based form of subtractive hybridization, representational difference analysis, to clone fragments of transcripts enriched in the proliferation zone. Of the 27 fragments obtained from the subtraction, 16 proved to be enriched in the proliferation zone when assessed individually by RNA dot blot. Clones of these fragments include an embryonic serine protease, a trypsin-like serine protease, a serine protease inhibitor, an antibacterial protein, several cuticle proteins, and 8 clones with novel sequences. In situ hybridization is currently being used to determine which cells express the corresponding transcripts. Supported by NIH grants DC02366 to TSM and DC00312 to CDD.

SERINE PROTEASES IN OLFACTION: THEIR FUNCTIONAL EXPRESSION IN THE OLFACTORY ORGAN OF SPINY LOBSTERS

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Serine proteases and their inhibitors are involved in many aspects of the development of nervous systems (Monard 1988 TINS 11:541), though little is known about their role in olfaction. Several serine proteases and inhibitors have been identified in the olfactory organ (antennule) of lobsters (Levine et al 2001 J Neurobiol 49:277; Stoss et al 2001 Chem Senses 26:1056; Hollins et al 2000 Chem Senses 25:646). One of these is a CUB-serine protease (Csp), expressed specifically in olfactory sensilla (aesthetascs) of the spiny lobster *Panulirus argus* (Levine et al., 2001). Csp appears to be secreted and associated with the membrane of olfactory receptor neurons, and is a homologue of trypsin-like serine proteases. In this study, we examine the function of Csp. First, we show that aesthetascs have trypsin-like protease activity, using a spectrophotometric assay with substrates and inhibitors of various classes of serine proteases. Second, to determine whether Csp is responsible for this trypsin-like activity, we expressed the Csp clone in cultured BHK cells, and tested for Csp activity using an immunoprecipitation assay and the spectrophotometric enzyme assay. Our results with this heterologous expression system suggest that this lobster olfactory Csp has trypsin-like activity. These studies are consistent with a role of Csp in development, although other functions (such as perireception) are also possible. Supported by NIH DC00312 and NSF IBN-0077474

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NOTCH SIGNALING GENES ARE COEXPRESSED WITH TASTE RECEPTOR CELL MARKERS, SUGGESTING DISCRETE LINEAGE RELATIONSHIPS.

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Mammalian taste buds are complex, multicellular receptor organs, comprising numerous immunocytochemically recognized cell types. Taste cells within buds have a brief lifespan, and are constantly replaced by basally located, proliferative stem cells. Recently, Stone and colleagues (submitted) have shown that each taste bud has between 7-12 of these stem cells, and have suggested that individual stem cells are lineage restricted, i.e., produce only one or a few types of taste receptor cells. To explore these ideas further, we have performed a series of gene and taste cell marker co-expression studies where *in situ* hybridization with antisense riboprobes for *Mash1* and *Dll-1*, members of the Notch signaling cascade typically involved in cell fate decisions, was combined with immunocytochemical localization of markers of taste receptor cells. In double label experiments on circumvallate taste buds of adult mice, *Mash1* appears to mark type III taste cells, whereas *Dll-1* expression is likely restricted to type II cells, as assessed by immunocytochemistry (Yee et al. 2001). These data suggest that *Mash1* and *Dll-1* are expressed in separate subpopulations of taste receptor cells. Given that *Mash1* and *Dll-1* are involved in cell lineage decisions in other systems, we now plan to test if these Notch signaling genes regulate genesis of discrete cell types within murine taste buds. Supported by NIDCD, DC03128, DC03947 & P30 DC04657.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF A TAURINE-SYNTHESIZING ENZYME IN THE RAT OLFACTORY BULB

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Taurine (2-aminoethanesulfonic acid) is richly present in the mammalian brain, and cysteine sulfinic acid decarboxylase (CSD) is the rate-limiting enzyme of its biosynthesis. Taurine has been proposed to be a modulator of synaptic transmission as well as a trophic factor essential for neuronal differentiation. The main olfactory bulb contains the highest levels of taurine among the brain regions studied. Moreover, taurine is the most abundant neuroactive amino acid in the olfactory bulb, exceeding glutamate and GABA in concentration. An immunohistochemical approach, with antiserum against CSD purified from rat liver, has been applied to investigate the nature of taurine-producing cells in the brain. These studies localized CSD to astrocytes in the cerebellum and hippocampus, implying that brain synthesis of taurine is solely associated with glial cells. We used polyclonal antibodies to a synthetic fragment of the CSD protein to examine the distribution of the taurine-synthesizing enzyme in the rat olfactory bulb. CSD immunoreactivity was primarily located in mitral cells, numerous juxtaglomerular neurons, and axons of olfactory sensory cells. These elements also showed immunostaining for taurine. The results demonstrate potential sites of taurine production in the olfactory bulb and suggest that neuronal cells are involved in taurine biosynthesis in the brain. Supported by NIH grant DC04083.

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CHARACTERIZATION OF OUTPUT CELLS IN THE ZEBRAFISH OLFACTORY BULB.

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The goal of this study was to characterize the morphology of the output neurons in the olfactory bulbs of mature zebrafish. Cells were labeled using a modified Golgi-Kopsch technique to illustrate whole-cell morphology. In addition, retrograde tract-tracing using DiI placed in the medial and lateral olfactory tracts allowed visualization of output neurons. Whole bulbs or sections were viewed on a confocal microscope to identify the three-dimensional structure of these cells. A small subset of Golgi-labeled cells have a large cell body and thick dendritic processes with many branches and spines. These cells are localized to the outer rim of the olfactory bulb and appear to be the mitral/tufted cells. The dendritic arborizations of these cells are confined to one or a few adjacent glomeruli. A separate category of neurons was labeled with DiI placed in the olfactory tract. These cells are bipolar and do not appear to possess dendritic tufts that are characteristic of mitral/tufted cells. Further studies are necessary to better characterize and identify these cells. We are closer to understanding the variety of cell types in the zebrafish olfactory bulb. This will provide important information for other studies on the zebrafish olfactory system, including studies of the effects of deafferentation on the morphology of output neurons. This project was supported by the NIH-NIDCD (R15 DC04262-01A1) and the Lee Honors College of WMU.

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OLFACTION AND CONNECTIONS BETWEEN THE EPITHELIUM AND OLFACTORY BULB IN 3-METHYL INDOLE-TREATED MICE

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3-methyl indole (3MI) is a known olfactory epithelial toxin in rats but its effects in mice have not been examined previously. We used anterograde transport of horseradish peroxidase applied to the epithelium and the vomeronasal organ of Swiss Webster albino mice to assess epithelial projections to the olfactory bulb after intraperitoneal injections of 250 -350 mg/kg 3MI dissolved in vegetable oil. This treatment resulted in the absence of all reaction product in main olfactory bulb glomeruli for 8 or more days. Essentially all glomeruli of the accessory olfactory bulb were filled with dense reaction product. In olfactometric tests, behaviorally trained mice failed to detect a relatively high concentration (5%) of ethyl acetate vapor for 8 or more days after treatment. Mice treated with 200 mg/kg 3MI were anosmic for ~5 days. Our study indicates that treatment with 3MI produces a dose and time dependent disruption of olfactory input to the main olfactory bulb of mice while leaving input to the accessory olfactory bulb largely or entirely intact. Olfactometric tests confirm anosmia in the absence of input to the main olfactory bulb and indicate that detection of ethyl acetate vapor is not mediated by the accessory olfactory system.

THE MUSHROOM BODIES OF THE SCARAB BEETLE PACHNODA MARGINATA

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Reduced silver (Bodian) staining of the brain of the African fruit chafer *Pachnoda marginata* reveals prominent mushroom bodies (MBs), each with two broad disc-like calyces supplying a pedunculus that extends forwards in the protocerebrum to provide a vertical and a medial lobe. Each MB is composed by four hemicalyces whose Kenyon cell (KC) axons run in four separate bundles down the pedunculus and into both lobes. Golgi impregnations show that the KC axons are arranged semi-concentrically within each bundle. Each of the two lobes reflects this quadripartite structure. As in other neopteran insects, collaterals from antennal lobe projection neurons supply the calyces. Lucifer yellow fills show that olfactory input to the calyces from an antennal lobe projection neuron distributes to all four hemicalyces. KCs appear to comprise at most two morphological types and there is no apparent division of the calyces into discrete zones, as in honeybees and cockroaches. Restricted regions of the lobes are supplied by protocerebral afferents and supply few but large efferent neurons. Generally, these arrangements suggest a morphologically simple mushroom body. We thank Mr. Michael Zimmerman (BS) for technical assistance and the Stiftelsen för Internationalisering av svensk forskning (STINT) for financial support.

SELECTIVE RESPONSE TO CHEMOSENSORY STIMULI IN MEDIAL AMYGDALA

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In male hamsters, much chemosensory information about biologically relevant natural stimuli is detected via receptors in the vomeronasal organ. Information is passed initially to the accessory olfactory bulb (AOB), then to the anterior medial amygdala (MeA), which projects heavily to the posterior medial amygdala (MeP). The pathway from MeA provides the majority of the chemosensory input to MeP. Previous experiments have demonstrated that artificial stimulation restricted to the accessory olfactory system increased neuronal activity in MeA but not in MeP, as measured by increased Fos expression. In new experiments, naive male hamster were exposed to 2 socially relevant stimuli:- female vaginal fluid (HVF) and male flank gland secretion (FGS) and 2 biologically but not socially relevant stimuli - mouse and cat urine. We measured Fos expression in the rostral and caudal AOB (as defined by g-protein expression), as well as in MeA and MeP. HVF activated rostral and caudal AOB equally. FGS from non-cage mate male hamsters preferentially activated the rostral AOB. Males exposed to urine from male mice or from cats also had more Fos in the rostral than caudal AOB. These patterns of activation do not correlate with activation in more central regions- all stimuli activated MeA, but only the socially relevant (HVF and FGS) stimuli activated MeP. These data are consistent with anatomical evidence indicating convergence of rostral and caudal AOB input at the level of the medial amygdala but they suggest that the amygdala may sort and process chemosensory information according to different criteria than the AOB. The MeP has abundant steroid receptors and many social behaviors are dependent on an adequate steroid level. Supported by NIDCD T32-DC00044.

EVIDENCE FOR SEGREGATION OF FUNCTION WITHIN THE HAMSTER AOB

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Within mammals, the accessory olfactory system has been shown to be chemoarchitecturally heterogeneous. Two families of vomeronasal organ receptors have been identified based upon genetic sequence and the G-protein that they express. In several species that have been studied, the two families of receptors project differentially to the rostral or caudal half of the AOB. This anatomical separation has been demonstrated in several species with different histochemical stains, and based on findings by this and other labs, is thought to represent a functional separation. One of the more commonly used stains to demarcate the separation within the AOB is NADPH-diaphorase staining, a histochemical stain for NADPH, an electron donor in reactions synthesizing nonclassical neurotransmitters such as nitric oxide (NO). Davis (1991) described NADPH-diaphorase staining in the hamster and rat, and suggested that the glomerular layer of the hamster AOB stains uniformly with this marker. Our current findings demonstrate a reliable separation of rostral and caudal AOB using a modified NADPH-diaphorase staining protocol. We are using this stain as a marker for segregation of neurochemical processes to analyze the expression of c-Fos in the AOB in response to social odors. Male hamsters show significantly greater expression of c-Fos within the rostral half of the AOB in response to vaginal secretion from females. These data suggest that the rostral AOB of male hamsters may be specialized for the processing of signals relevant to sexual behavior and/or sexually relevant neuroendocrine responses. A description of the NADPH-diaphorase staining pattern as well as preliminary c-Fos data will be presented.

G-PROTEIN COUPLED RECEPTORS IN THE OLFACTORY SYSTEM: A STRONGLY CONSERVED MECHANISM OF SIGNAL TRANSDUCTION

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Olfactory signal transduction starts by the binding of odorant molecules to receptor proteins located on the cilia of olfactory receptor neurons. The heterotrimeric GTP-binding protein Golf, is part of the signal transduction cascade for odors that stimulate cAMP production. The animal model for this study, the sea lamprey, is a jawless vertebrate that represents over 400 million years of evolution separate from the main vertebrate line and a unique life history involving a radical transformation. Olfactory processes that are common to lampreys and other vertebrate species are likely to be conserved characteristics, and fundamental to the function of the vertebrate olfactory system. We used immunohistochemical and immunoblotting techniques to test for the presence of Golf in the lamprey. A 45 kD protein, immunoreactive to Golf was present in the ciliary fraction of the olfactory epithelium. In all developmental stages Golf was present in dorsal, anterior and ventral olfactory bulb glomeruli, but absent from medial glomeruli. We confirmed these results with double labeling experiments, staining for olfactory receptor neurons with GS1B4 lectin, and by anterograde labeling with fluorescent dextran. Demonstration of Golf immunoreactivity in the lamprey olfactory pathway, suggests a high degree of conservation of this protein during vertebrate evolution. The clear separation of medial non-Golf expressing olfactory receptor neurons from Golf expressing neurons in the remaining glomeruli, suggests the presence of an independent pathway for odor coding in the medial olfactory bulb of the lamprey. Supported by NSERC (BZ) and by the Great Lakes Fishery Commission (WL)

BEX-OMP INTERACTION

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Evidence for OMP involvement in the olfactory sensory transduction cascade derives from our recent demonstrations of electrophysiological and behavioral deficits in the OMP-null mouse (Ivic et al. 2000, Youngentob et al. 2001). We recently identified Bex proteins as novel interactors with OMP. The Bex proteins exhibit several functional motifs including (1) a site for binding transition elements; (2) cysteines for intra- or inter- molecular interaction; (3) a Ca²⁺ /CaM kinase II phosphorylation site; and (4) a conserved motif for isoprenylation to target proteins to the membrane. This provides a potential link between cytoplasmic OMP and its ability to modify events of olfactory transduction. Bex cDNA was isolated through T7 phage-display screening using OMP as a bait. In vitro and in vivo interactions between OMP and Bex were demonstrated by chemical crosslinking, NMR, and solid phase binding assays. To study the interaction of OMP and Bex in ORNs, and to identify the site of co-localization, we generated rabbit antisera against the full length Bex1 and chicken antisera against a synthetic Bex peptide. These antisera show specificity on immunoblots and will be used for immunoprecipitation assays and immunocytochemical localization of Bex proteins in olfactory and non-olfactory tissues. These antisera facilitate immuno-labeling experiments for evaluating localization, interaction and translocation of these proteins in response to specific manipulations. Supported by NIHDCD03112 (FLM)

CLONING GUANYLYL CYCLASE ACTIVATING PROTEINS FROM THE OLFACTORY SYSTEM OF MANDUCA SEXTA

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Regulation of cGMP levels is important for long-term adaptation of olfactory receptor neurons. We are studying the regulation of cGMP in the olfactory system of the moth *Manduca sexta*. We previously cloned and characterized a novel guanylyl cyclase (MsGC-I), which is strongly expressed in the cell bodies and dendrites of olfactory receptor neurons. MsGC-I has a catalytic domain with sequence similarity to receptor guanylyl cyclases (rGCs), but contains no ligand-binding, transmembrane, or kinase-like domains. Guanylyl cyclase activating proteins (GCAPs) are small, calcium-binding proteins which regulate the activity of rGCs. To investigate whether the activity of MsGC-I could be regulated by GCAPs, we used degenerate oligonucleotide RT-PCR to identify fragments of two GCAP-like molecules from antennal cDNA. One fragment (MsGCAP-I) has 69% sequence identity with a neuronal calcium sensor protein (NCS-2) cloned from *Caenorhabditis elegans* and the other fragment (MsGCAP-II) has 98% sequence identity with *Drosophila melanogaster* frequenin. The presence of GCAP-like molecules in the olfactory system suggests they might regulate the activity of MsGC-I or other rGCs in *Manduca sexta*. Supported by National Institutes of Health grant DC04292

CHARACTERIZATION OF A DISTINCT CHEMOSENSORY SUBSYSTEM IN THE MAMMALIAN NOSE

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To detect and encode odors and pheromones, the mammalian nose uses anatomically distinct neuronal subpopulations (e.g., olfactory receptor neurons, vomeronasal neurons) that exhibit different stimulus sensitivities, receptors and transduction mechanisms, and project to different areas of the brain. One of these subpopulations is a group of ciliated neurons (D neurons or DN) that express the membrane guanylyl cyclase GC-D and project to the "necklace" glomeruli (NGs) in the caudal olfactory bulb (OB). We have begun to characterize the anatomy and functional role of this group of cells and glomeruli, which may comprise a distinct chemosensory system of unknown function. By gene targeting, we disrupted the mouse *GC-D* gene and inserted a *tau-lacZ* reporter into the *GC-D* locus. Beta-gal-positive DN project along the ventrolateral aspect of the OB, innervating the NGs between postnatal days 1 and 2; this innervation pattern is maintained throughout development. Tyrosine hydroxylase immunohistochemistry, a correlate of afferent activity, is largely absent in NGs of *GC-D* ^{-/-} mice. Measurements of survival and weight gain by newborn mice show no differences across *GC-D* genotypes, disproving the hypothesis that DN are required for successful suckling. These results indicate that GC-D plays a central role in DN function, and suggest that DN and NGs are components of a distinct chemosensory subsystem. Supported by NIDCD (SM, RR), HHMI (DG, RR).

TRANSGENIC ANALYSIS OF THE MOUSE GUANYLYL CYCLASE-D PROMOTER

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Expression of olfactory-specific guanylyl cyclase-D (GC-D) is restricted to a small population of olfactory sensory neurons which appear to be randomly dispersed within a single topographic zone in the main olfactory epithelium (Fülle et al., 1995). Coexpression studies suggested that this receptor guanylyl cyclase may function in a unique cGMP-signaling pathway. Neurons expressing GC-D project to a distinct group of glomeruli in the olfactory bulb (Juilfs et al., 1997) that are thought to process olfactory cues associated with suckling behavior in neonatal rodents. Previously, we characterized the overall genomic organization of the mouse GC-D gene (*Gucy2d*) including its 5'-untranslated region which is interrupted by one intron. We identified potential promoter and transcriptional regulatory motifs including olfactory-specific motifs like Olf-1 and NF-1 binding sites. To delineate sequences that confer the specific spatial expression pattern of GC-D, we now began to analyze the *Gucy2d* promoter *in vivo*. We fused fragments of the *Gucy2d* 5'-flanking region to the reporter genes lacZ or GFP and microinjected linearized promoter-reporter constructs into fertilized mouse eggs in order to generate transgenic founder progeny. Tail DNA from founders were screened for exogenous transgenes by PCR and Southern blot analyses. Transgenic mice bearing the *Gucy2d* promoter-reporter constructs have been obtained. Further analysis of the transgenic models should help to identify promoter regions that are important for the specific spatial expression pattern of GC-D and to understand regulation of olfactory receptor expression in general at the *in vivo* level. Supported by NIH grant DC04281 to HJF.

REGULATION OF CYCLIC AMP IN THE CILIARY CYTOPLASM OF THE OLFACTORY RECEPTOR CELL

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We investigated the effect of cNMP on the cytoplasm of cilia in the newt olfactory receptor cell. In this experiment, we applied caged cNMP to isolated living olfactory receptor cells with the whole-cell patch clamp method. Photolysis of caged compounds were controlled quantitatively by UV light stimulation locally applied to the cilia. Light illumination induced an inward current in all tested cells which were loaded with either 1mM caged cAMP or 1mM caged cGMP. The amplitude of the light-induced current was dependent on both light intensity and duration. The intensity- and duration-response relation were well fitted by the Hill equation with high cooperativity (Hill coefficient, approximately 4-5), supporting the notion that Cl current added onto cNMP-induced current makes a non-linear booting of the signal transduction. To confirm that idea, we examined Hill's fitting for developing phase of the current at +100mV and -50 mV. The developing phase became more slowly at +100 mV, and the time course was fitted by a smaller Hill coefficient. Furthermore, we compared light- and odorant-induced response, to estimate the activation time course of adenylyl cyclase. Adenylyl cyclase was activated about 260ms later from the onset of the odorant stimulation. When long steps (light and odorant) were applied to cells, odorant-induced current showed stronger and more remarkable decay than the light-induced response. This observation suggests that the molecular system regulating desensitization locates upstream of the cAMP production site in addition to the direct modulation of CNG channel.

EFFECTS OF CYCLIC NUCLEOTIDES AND BIOGENIC AMINES ON OLFACTORY SENSILLA OF THE HAWKMOTH MANDUCA SEXTA.

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Previous experience of strong odor stimuli as well as biogenic amines modulate the sensitivity of insect olfactory sensilla (OS) via mechanisms involving cyclic nucleotides. To examine cyclic nucleotide- and amine-dependent modulation of OS, we performed tip recordings of pheromone-dependent sensilla of the moth *Manduca sexta*, as well as patch clamp recordings of their cultured olfactory receptor neurons (ORNs). In extracellular tip recordings of unstimulated pheromone-sensitive OS the transepithelial potential (TEP) oscillated regularly with periods of 2-6 min and 1-25 mV peak-to-peak amplitudes during intervals of several hours. Octopamine injections reduced the amplitude of the TEP oscillations and the resistance of the sensillum preparation, while serotonin affected only the waveform of the TEP, apparently via effects on supporting cells. Injections of 8-bromo-cGMP did not mimic amine effects but appeared to change the waveform of spontaneous action potentials of ORNs. Application of cGMP in whole-cell patch clamp recordings blocked one type of delayed rectifier potassium and a non-specific cation channel, while activating another cation channel. We hypothesize that the rises in cyclic GMP-concentrations after strong pheromone stimuli block Ca⁺⁺-impermeable protein kinase C-dependent cation channels, while activating Ca⁺⁺-permeable cation channels. Thus, adapting stimuli cause smaller depolarizations together with long-lasting increases in intracellular Ca⁺⁺-concentrations and a decrease of the action potential response. Supported by DFG grants STE 531/10-1,2,3.

CAMP-INDEPENDENT AND CAMP-DEPENDENT RESPONSES OF OLFACTORY NEURONS IN XENOPUS LAEVIS TADPOLES

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We report on responses of olfactory receptor neurons (ORNs) upon application of amino acids and forskolin using a novel slice preparation of the olfactory epithelium of *Xenopus laevis* tadpoles. Both amino acids and forskolin proved to be potent stimuli. Interestingly, a substantial number of ORNs that responded to amino acids did not respond to forskolin. This suggests that some amino acids activate transduction pathways other than the well-known cAMP-mediated one. The differential processing of cAMP-mediated stimuli on one hand and amino acid stimuli on the other was further elucidated by Ca²⁺-imaging of mitral cells in the olfactory bulb. The projection pattern of amino acid-sensitive ORNs to mitral cells differed markedly from the projection pattern of forskolin-sensitive ORNs. Mitral cells activated by amino acids were located laterally compared to those activated by forskolin, and only a small proportion responded to both stimuli. We therefore conclude that sensory transduction of a number of amino acids is cAMP-independent, and amino acid- and forskolin-mediated responses are processed differentially at the level of the olfactory bulb.

THE RELATIONSHIP BETWEEN THE ELECTRONIC / STRUCTURAL PROPERTIES AND THE ODOR ACTIVITIES OF PYRAZINE DERIVATIVES

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The characteristics of odorant molecules recognized by odorant receptors are still controversial, although numerous studies have been made on the molecular recognition of the olfactory sensation. To clarify the relationships between those characteristics and receptors; electronic / structural properties of the molecules, pyrazine and its derivatives, were examined in detail. These properties were investigated using molecular mechanics (MM3) and ab initio molecular orbital calculations. The odor activities were referred to the known data. The obtained results have suggested that the magnitude of the sphere of the lone-pair orbital on the nitrogen atom and the conformation of the substituent groups attached in the pyrazine ring are the essential properties that give rise to the activities. These two characteristics play a significant role in the ligand recognition step at the receptor site. In order to estimate these two characteristics quantitatively, we used two indices as electronic / structural descriptors. The regression equation based on these two descriptors and the activities from the known data was described. From the results of odor threshold measurements, the observed activities for the newly designed pyrazines could be predicted by the equation. Furthermore, to confirm that those pyrazines interact with the common receptors, threshold measurements for the two admixed pyrazines, one with low activity (p-l) and the other with high activity (p-h), were performed. The threshold of mixture (p-h & p-l) was higher than that of p-h only. Those experimental data suggested that those pyrazines are recognized by the common receptors.

DISTRIBUTION OF NERVE FIBERS IN THE SEA CATFISH PLOTOSUS LINEATUS BARBEL

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The sea catfish has four paired barbels of almost equal length which serve as the major taste and tactile organs. To reveal the neural organization of these taste and mechanosensory systems we examined the patterns of innervation in the barbels. Generally paired taste buds are located in the epidermis throughout the length of each barbel. The density of taste buds is low proximally and increases distally. Viewed in transverse section, taste bud density is highest rostrally, fairly high caudally and lowest in the intermediate zone between. The carbocyanine dye, DiI, was applied to the nerve stump in dissected, fixed barbel specimens to trace nerve fibers. The barbel nerves enter the caudal region at the base of the barbel as a trunk. Bundles of various sizes exit to innervate taste buds as the trunk courses towards the tip. As these bundles innervate the rostral and caudal epidermis they ramify repeatedly to make hexagonally-shaped networks under the epidermis; few networks were found in the intermediate zones. Rostral networks were smaller than caudal networks and both became smaller towards the apical region. Nerve strands exit each network to innervate taste buds. Each strand divides into two substrands, which enter the same bud basolaterally opposite one another. 20-50 strands originate from each network, thus a network innervates a total of 10-25 taste buds. Peri-gemmal and extra-gemmal fibers were also observed. The significance of peripheral networks and bifurcated innervation of sea catfish taste buds remains to be determined.

DEGENERATION OF TASTE BUDS IN MOUSE FUNGIFORM PAPILLA AFTER CHORDA-LINGUAL NERVE TRANSECTION

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Taste buds on the anterior two thirds of the tongue are innervated by the chorda tympani nerve. Disruption of the chorda tympani nerve results in the degeneration of taste buds in fungiform papilla. This phenomenon has been well documented in hamster, rat, and gerbils, but not in mouse. The present study looks at taste bud degeneration in fungiform papilla of the C57BL/6J mouse and provides baseline data for future investigations. Adult mice received unilateral chorda tympani-lingual nerve transection and were sacrificed 4-30 days after surgery. Tongues were sectioned and processed using either hematoxylin and eosin staining or immunohistochemistry for the taste receptor specific marker cytokeratin 8. Eleven days after transection, taste buds were still prevalent on the sectioned side of the tongue and contained cytokeratin 8 positive cells. By 22 days after chorda tympani-lingual nerve transection, almost no taste buds remained. Fungiform papillae were significantly fewer while more "filiform-like" papillae were observed relative to the intact side. On the side with nerve transection, only the very tip of the tongue seemed unperturbed; having normal taste buds and fungiform papilla. The ability to insert or remove genes makes the mouse a powerful model for studying mechanisms of nerve regeneration and receptor-nerve interactions. Genes of the C57CL/6J mouse have been well mapped and many mutants are readily available. These data provide a basis for future investigations taking advantage of these genetic manipulations. Supported by NIH Grant DC03576

SEMIQUANTITATIVE ANALYSIS OF ALTERATIONS IN INTRAGEMMAL NERVE FIBERS IN IRRADIATED TASTE BUDS LABELED WITH GAP/B50 OR SYNAPTIC VESICLE PROTEINS

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Radiation therapy produces alterations in taste which can cause a significant decrease in nutrition intake for patients undergoing radiation therapy. Despite the notion that neurons are radioresistant, damage to nerve endings may have a role in mediating the taste alterations. In general histologic preparations, intragemmal nerve fiber architecture appears preserved for several days following radiation exposure. However, semiquantitative analysis reveals alterations in the nerve fiber architecture at early time points. Using the Bioquant Image Analysis System (RNM Biometrics, Nashville, TN) the amount of immunolabeled nerve fibers was measured as the % of labeled pixels per total area (pixels) of taste bud. Nerve fibers were quantitated in animals receiving a single 1700 cGy exposure after 7 or 9 days survival and in control animals. Results indicate the average percent of nerve fibers in control animals labeled with GAP/B50 was 55.07% and labeled with SV2 was 21.52%. Fibers labeled with GAP/B50 at 7 days after radiation was 21.06% and at 9 days was 11.0%. These results indicate a decrease in either the length or in the number of nerve fibers within irradiated taste buds. This decrease begins prior to observable alterations in taste bud structure, as seen on routine histologic preparations, and prior to the loss of sensory cells. This dissociation between taste bud and nerve fiber alterations may be a possible mechanism for the taste loss seen prior to the loss of receptor cells in irradiated taste buds. NIDCD DC00166-02.

SIMULTANEOUS CHRONIC RECORDING FROM MULTIPLE SINGLE FIBERS OF THE CHORDA TYMPANI NERVE USING AN IMPLANTABLE SIEVE ELECTRODE ARRAY

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Over the past several years we have been developing a totally implantable electrode system to record from afferent taste fibers. The current device is fabricated from polyimide and consists of a diaphragm with a large number of holes some of which are surrounded by annular electrode sites. A ribbon cable is also fabricated with the diaphragm that connects the electrode sites to a percutaneous headcap housing containing a connector. The metal conductors are made from a titanium alloy and the electrode sites are gold plated. Once completed the assembly is tested for electrical continuity and the electrode impedances measured. Small diameter guide tubes are cemented to opposing faces of the electrode diaphragm. The assembly is then implanted between the cut ends of the rat chorda tympani nerve that innervates taste buds on the anterior two third of the tongue. After about 5-6 weeks to allow the chorda tympani fibers to regenerate through the holes in the diaphragm the rats are briefly reanesthetized and the external amplifying equipment connected via the headcap connector. Taste stimuli are flowed over the tongue. Currently successful recordings have been made from 4 implants all of which have 4 electrode channels. It is possible to make few fiber responses from all four channels for up to 10 days after which further fibers regenerate through the holes and the recordings becomes multiunit. Different patterns of responses across the four channels result from stimulation with different taste modalities. Supported by NIDCD grant DC04198 to RMB.

COMPARISON OF THE RESPONSES OF THE CHORDA TYMPANI AND GLOSSOPHARYNGEAL NERVES TO TASTE STIMULI IN C57BL/J MICE

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The purpose of the study was to characterize and compare the responses of whole chorda tympani (CT) and glossopharyngeal (NG) nerves to a wide array of taste stimuli in C57BL/J mice. Method: Inhalation anesthesia with either halothane or isoflurane was used. Recordings were carried out in 18 C57BL/J mice. In 13 animals responses from both the CT and NG were obtained. Results: There were statistically significant differences in the responses of the two taste nerves to sweet, bitter and umami stimuli. First, sweeteners prevailed in the CT. Thus sucrose, fructose, tryptophan, SC45647, NC00174, cyanosuosan and D-phenylalanine elicited stronger responses in the CT than in the NG. Responses to acesulfame-K, saccharin and L-proline did not significantly differ in two nerves. Second, bitter taste dominated in the NG. Amiloride, atropine, caffeine, cycloheximide, denatonium, L-phenylalanine, MgSO₄ and PROP were more effective in the NG than in CT. Chloroquine, however, elicited stronger responses in the CT than in NG. Responses to brucine, strychnine, TEA, QHCl, quinacrine and sparteine were not significantly different in two nerves. Finally, in regard to umami compounds MSG was more effective in the NG than in CT, while IMP elicited significantly larger responses in the CT than in NG. Conclusions: In C57BL mice the sensitivities of the two nerves were different to various compounds. Differences were found for stimuli belonging not only to different taste qualities, such as bitter and sweet, but for stimuli of the same taste quality. Thus the multiple mechanisms involved in sweet and bitter tastes are probably not distributed uniformly throughout the tongue.

ELECTROGUSTOMETRIC THRESHOLDS: RELATIONSHIP TO ANTERIOR TONGUE LOCUS, AREA OF STIMULATION, AND NUMBER OF FUNGIFORM PAPILLAE

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Chemical taste thresholds are inversely related to the number of fungiform papillae. It is not known whether a similar relationship exists for electrogustometric thresholds. We determined anodal electrical thresholds for 16 subjects at 4 left-side anterior lingual locations: tongue tip, a region 1.7 cm posterior to the tip, a region 3.4 cm posterior to the tongue tip, and a region 1.7 cm from the tip along the lateral margin. Two electrode sizes were employed (12.5 and 50 mm²), and stimulus duration was held constant at 0.5 sec. The number of fungiform papillae was established using videomicroscopy. Analogous to what is seen with chemical stimulation, an inverse relationship was present between the mean electrical thresholds, expressed in terms of current density, and the number of papillae within the stimulated regions. The tongue tip – which has the highest density of fungiform papillae -- was found to be more sensitive than the other tongue regions evaluated. Also paralleling chemical thresholds, the mean electrical threshold values were lower (i.e. sensitivity was higher) at a given tongue locus for the 50 mm² than for the 12.5 mm² stimulus area. Overall, this study demonstrates that thresholds for electrical stimulation vary across discrete regions of the anterior tongue and are specifically related to the number of fungiform papillae within the stimulated regions. These observations provide additional support for the hypothesis that lingual sensations induced by low levels of electrical current are mediated by the taste system. Supported, in part, by grants PO1 DC 00161, RO1 DC 04278, and RO1 DC 02974 and AG 17496 from the National Institutes of Health, Bethesda, MD USA.

ORAL PHANTOMS: EVIDENCE FOR CENTRAL INHIBITION PRODUCED BY TASTE

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Previous studies have suggested that taste input produces central inhibition such that loss of input (damage or anesthesia) can lead to intensified oral sensations as well as phantoms (sensations in the absence of stimulation). The central role of taste led us to evaluate patients presenting with any oral sensory phantoms. Patients seen include those with taste phantoms (N=21), oral burn phantoms (N=55, about half have taste phantoms as well; some patients were described at AchemS1999), atypical odontalgia (N=23, tooth pain in the absence of pathology; patients were described at the meeting of the American Academy of Oral Medicine 2001) and tactile phantoms (N=7). Taste was evaluated with a spatial taste test. Concentrated NaCl, sucrose, citric acid and QHCl were swabbed onto each side of the tongue stimulating fungiform (CN VII) and circumvallate (CN IX) papillae. Taste ratings were made with the general Labeled Magnitude Scale. Controls (N=64) were slightly older than patients; patients and controls were predominantly female. ANOVAs showed partial loss for QHCl on the anterior tongue for all patient groups. All but the tactile phantom group also showed losses for bitter (as well as some other tastes) at the circumvallate papillae. Clinically, many of these oral phantoms are reduced with the GABA agonist clonazepam. Either intrinsic brainstem circuits or descending forebrain pathways could account for the phenomena. Anatomical and electrophysiological evidence supports both possibilities. Supported by NIDCD 00283.

PEROMONAL SIGNALS IN A TANGERINE-SCENTED SEABIRD

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Among vertebrates, the lack of evidence for pheromones in birds is surprising, as every bird examined has a functional olfactory system, and some produce unusual odors. The crested auklet (*Aethia cristatella*), a monogamous seabird, exhibits a distinctive, tangerine-like scent closely associated with courtship. Using T-maze experiments, we established that crested auklets: 1) preferred conspecific feather odor (n=34, t = 2.9, P = 0.007), 2) were attracted to two seasonally-elevated, tangerine-scented chemicals identified in feather odor (cis-4 decenal and octanal; n=49, t = 3.8, P = 0.0004), and 3) actively discriminated between odors (F = 8.05, P = 0.0005), as indicated by attraction to auklet scent (above), aversion to mammalian musk (n=35, t = -2.4, P = 0.02), and no significant response to a novel cue, banana essence (amyl acetate; n=46, t = -0.03, P > 0.9). Our data, combined with the striking relationship during mutual mate choice between feather odor and 'ruff sniff' courtship displays, indicate that crested auklets employ pheromones during breeding. Although the social function of odors is just beginning to be realized in birds, such as crested auklets, further studies promise to reveal the more widespread use of chemical communication in avian species. Research was funded by NSF and NSERC grants to JCH and ILJ, respectively. Partial support for chemical analyses (LELR) was provided by Biospherics Research Corporation.

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UNUSUAL PHEROMONE RECEPTOR NEURON RESPONSES IN HELIOTHINE MOTH ANTENNAE DERIVED FROM INTER-SPECIES IMAGINAL DISC TRANSPLANTATION

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In moth species that utilize sex pheromones for mate location, males possess olfactory receptor neurons (ORNs) on their antennae that differentially respond to behaviorally agonistic and antagonistic pheromone components. Pheromone specific ORNs in sympatric heliothine species, *Helicoverpa zea* and *Heliothis virescens* are housed in long sensilla trichodea. Both species share the same major sex pheromone component but have different minor components and behavioral antagonists. We used the technique of transplanting larval antennal imaginal disks between males of the two species. By using single-cell electrophysiological recording technique, we investigated the specificities of the ORNs of the transplanted antennae in adult moths. The majority (60-65%) of ORNs sampled from males of either type of cross-specific transplants had response characteristics consistent with those of the donor, not the recipient species. Similarly, the majority of moths flew upwind more often to the donor species' pheromone blend than to the blend of the recipient species in a wind tunnel bioassay. However, we also found some unusual response characteristics not seen in normal, untransplanted antennae of either species. About 20% of ORNs sampled in either type of transplants responded like those of the recipient species. We also discovered in both species ORN types that were not typical of either species. Our data suggest that tissue on the recipient animal is capable of exerting influence over the types of ORNs that grow out of the transplanted antennal imaginal discs. Supported by NSF grant #IBN9910783 to T.C. Baker.

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SEX DIFFERENCES IN OLFACTORY CROSS-ADAPTATION OF HUMAN SWEAT ODOR

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Repeated sniffing an odorant results in adaptation. This process can sometimes affect sensitivity to other odorants (cross-adaptation). We focused upon the ability of different types of odorants (53 altogether) to cross-adapt malodorous axillary secretions. In Experiment I, 16 volunteers (8 females) sniffed 41 odorants, each for 2.5 min. Every 30 sec during this adaptation phase, stress-derived axillary odors from males were introduced and rated for intensity. When compared against baseline levels, significant cross-adaptation of the axillary odor was noted for 8 odorants. Data for males and females were then evaluated independently. Baseline intensity and pleasantness ratings of the odorants and the axillary odor did not differ between males and females; however, none of the odorants produced satisfactory cross-adaptation in females. In Experiment II, we attempted to cross-adapt female axillary odors with many of the 41 odorants plus others; some produced cross-adaptation and others did not. This time, however, more of the odorants produced greater cross-adaptation in females than in males. These results reveal unexpected sex differences in the production of axillary odors and, importantly, sex differences in the perception of these odors, especially in the presence of other, competing odorants. It is as if the biologically relevant odor from the opposite sex appears to break through. Supported in part by NIH DC000298 and grants from Haarmann & Reimer.

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EFFECTS OF BREASTFEEDING CHEMOSIGNALS ON HUMAN SEXUAL MOTIVATION.

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Chemosensory signals from the breastfeeding environment modulate the physiology and behaviors of conspecifics in many species, including humans. The present randomized, double-blind study aimed to assess the effects of such chemosignals on the sexual motivation of other women. To this aim, axillary and breast secretions were collected from 26 lactating women whose infants were exclusively breastfeeding. These pads contained mothers' body odors, milk, and, likely, compounds from infants, as mothers nursed several times during each collection period. Forty-seven, nulliparous women were randomized to two recipient groups. After daily exposure to control pads containing buffer solution during a baseline cycle, those in the experimental group were exposed to pads worn by unfamiliar lactating women, whereas those in the control group continued to be exposed to the control pads, throughout the two subsequent cycles. Although the groups did not differ during baseline, women exposed to breastfeeding pads reported significant increases in sexual desire and fantasies. The frequency of sexual activity, likely constrained by a sexual partner, was not affected by chemosignal exposure. However, women with partners reported more desire for sexual intimacy, while those without reported significantly more fantasies, compared to their respective controls. Thus, breastfeeding chemosignals increased women's sexual motivation, but did so in different ways, depending on the context created by having a regular sexual partner.

176 Symposium The Presidential Symposium: The Chemicals of the Chemical Senses

INTRODUCTION: THE CHEMICALS OF THE CHEMICAL SENSES

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The chemical sense organs are the best detectors of environmental chemicals on the planet. As the "molecular" sense it is critical to understand the chemistry behind these olfaction and taste molecules? How does chemistry relate to chemoreception? Can we find new odorants and tastants? Is it possible to finally arrive at a rational and useful classification of odorant and tastant molecules? This symposium will also include scientists from the related fields of medicinal chemistry and pharmacology and combinatorial chemistry. An introductory talk will be given by the world renowned English physical chemist and author of several popular books and University texts, Peter Atkins.

177 Symposium The Presidential Symposium: The Chemicals of the Chemical Senses

MAKING MOLECULES MATTER

Atkins P.¹ ¹*Lincoln College, University of Oxford, Oxford, UK*

In this very general, introductory lecture on everyday molecules, I will explore the everyday world and look at some of the molecules that are responsible for its wonderful richness. I will also trace the connection between structure and properties of the molecules in everyday objects and illustrate how a chemist thinks about the world. I will start with detergents, fabrics, and polymers and explore how the physical properties and in some cases electrical properties of polymers arise from their structure. I shall then move on to the senses, where molecular recognition is at the root of sensation. In this section I will talk about some of the molecules responsible for taste, odor, pain, and hallucination. Vision is also, in its mechanism, a chemical sense, and I will talk both about the molecular mechanism of vision and also the molecules responsible for color in the natural world. The lecture will be entirely nontechnical, and is intended in part to set the scene for the following presentations. It is also intended to be an exercise in showing how that most maligned of sciences, chemistry, can be really enjoyable and open up a deeper vision of the world.

178 Symposium The Presidential Symposium: The Chemicals of the Chemical Senses

USE OF HIGH THROUGHPUT DISCOVERY TECHNIQUES TO CREATE NOVEL FLAVOR AND FRAGRANCE MOLECULES

Rogers D.H.¹ ¹*Associate Director, Synthetic Chemistry, Senomyx, La Jolla, CA*

Over the past ten years developments in the areas of combinatorial chemistry, high throughput screening, and informatics has allowed the chemist to synthesize, screen, and track more molecules than ever before. Combinatorial chemistry, initially embraced and utilized by the pharmaceutical industry, provides the capability to create large chemical libraries in very short time periods. From its inception over a decade ago, the field has changed dramatically. Initially focusing on the rapid generation of peptide arrays on solid support, in more recent times the field has gravitated toward the synthesis of a variety of non-peptide, low molecular weight, and more diverse organic compounds. These developments coupled with recent developments in gustatory and olfactory receptor biology provides all the necessary pieces for a high throughput discovery process focused on creation of new flavor and fragrance molecules. This paper will describe such a process, one that tightly integrates combinatorial chemistry, automated analytical and purification techniques, informatics and high throughput screening with a focus on discovering new compounds for taste and smell.

179 Symposium The Presidential Symposium: The Chemicals of the Chemical Senses

COMPUTATIONAL METHODS IN STRUCTURE-BASED ODORANT DISCOVERY

Richards N.G.¹ ¹*Department of Chemistry, University of Florida, Gainesville, FL*

The high-resolution crystal structure of rhodopsin is revolutionizing the development of new drugs by allowing the use of homology modeling methods to construct models of cellular G protein-coupled receptors (GPCRs). In principle, the rhodopsin structure also provides a template for developing models of odorant receptors that can be used to understand how odorants interact with their cellular targets, a key element in the discovery of new classes of odorants using rational pharmacophore-based strategies. In practice, the combinatorial mechanisms that appear to underpin odorant receptor signaling and odor perception raise intriguing questions about whether the techniques used in rational drug discovery can be applied to creating useful structure-activity relationships for odorants. This lecture will provide an introductory overview of computational methods for (i) modeling odorant receptors, (ii) docking ligands into the transmembrane domains of these structures, and (iii) developing structure-activity hypotheses. The likely limitations of these theoretical approaches, and their integration into experimental studies will also be discussed.

180 Symposium The Presidential Symposium: The Chemicals of the Chemical Senses

DESIGNING FRAGRANCE INGREDIENTS

Sell C.¹ ¹*Quest International, Ashford, Kent, England*

Synthetic organic chemistry has revolutionised perfumery over the last hundred years. Many of the major ingredients of today were unknown at the beginning of the twentieth century. These new generation materials provide advantages over their natural predecessors in terms of performance in hostile product media, cost, availability, security of supply and additional functional benefits. The search for new materials continues in the light of the ever increasing demands being made of fragrance ingredients. These demands may conflict with each other; for example, a requirement for stability to hypochlorite bleach coupled with the need to degrade rapidly in the environment. The construction and refining of statistical structure/property correlation models plays a valuable role in the design of new, improved perfume ingredients. Odour is, of course, one of the properties for which such models exist. In some odour areas, such as camphor and sandalwood there are models with good predictive power for character. Character is much more difficult to predict in some other odour areas and there is no good predictor for intensity. There is no universal structure/odour correlation model since there are known exceptions for every one which has been proposed. It is very tempting for the chemist to build models of the process of olfaction based on the more successful statistical structure/odour models. However, there are intellectual traps inherent in doing so and one must be aware of these. Experimental fact must always take precedence over intellectual theory

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MOLECULAR PERFORMANCE AND PERCEPTION INFORMATICS

Brooks C.D.¹ ¹*VP Research and Development, International Flavors and Fragrances, Union Beach, NJ*

The challenge to create informatic systems that collect and model physical/chemical, perceptual and emotional properties of flavor and fragrance molecules will be discussed. Chemoreception remains a frontier of science due to its complexity and multidisciplinary nature. Molecular perception defies digitization, as it is more than a simple on/off switch. Thousands of flavor and fragrance molecules are known and many more are constantly being discovered from natural sources and by chemical synthesis. The ability to create predictive models that relate chemical structure to perceptual properties requires a multidisciplinary approach with contributions from both academic and industrial research. Receptor based biochemical assays offer molecular level data that can be compared to human sensory data. Improved methods of acquiring and applying sensory data provide an important contribution to consumer product development.

182 Poster Neural Plasticity and Regeneration

PLASTICITY UNDERLYING ANDROSTENONE LEARNING MAY BE MEDIATED CENTRALLY RATHER THAN PERIPHERALLY

Mainland J.D.¹, Bremner E.A.², Young N.¹, Johnson B.N.³, Sobel N.¹

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The olfactory system is a model for adult neural plasticity. An example is "androstene learning." Approximately 30% of the adult human population does not perceive an odor when sniffing androstene, but sensitivity can be induced by repeated exposure (Wysocki et al., 1989). This acquired ability to detect an odor suggests plasticity and learning at some level of the olfactory system. Here we present data suggesting that this plasticity may involve central, rather than peripheral components of the olfactory system. We screened 130 subjects using a three-alternative-forced-choice paradigm. Of these, 38 were non-detectors (29%). To date, 12 non-detectors completed a 21-day regimen of monorhinal androstene exposure. Exposure did not induce sensitivity at levels previously reported, but did significantly increase overall detection accuracy for crystal androstene from chance to 22% above chance in the exposed nostril ($p < .002$), and 16% above chance in the unexposed nostril ($p < .02$). There was no significant difference in the extent of improvement between the exposed and unexposed nostril ($t(11) = .4$, $p = .7$). A group of 8 untrained control subjects remained at chance ($p = .4$) at the end of an identical period of time. These preliminary data indicate that plasticity involves components of the olfactory system receiving birhinal input. Funding provided by Searle fellowship, SOSI and NIH-NIDCD

183 Poster Neural Plasticity and Regeneration

ODOR STIMULATION MODULATES APOPTOSIS IN ADULT OLFACTORY (PIRIFORM) CORTEX OF THE RAT

Leung C.H.¹, Wilson D.A.¹ ¹*Department of Zoology, University of Oklahoma, Norman, OK*

Ontogeny and maintenance of normal anatomy and synaptic physiology of the olfactory cortex requires normal sensory input. For example, reduced levels of odorant stimulation during development can reduce dendritic complexity of semilunar pyramidal neurons in the anterior piriform cortex of the rat (aPCX; Wilson, Best & Brunjes, 2000). These same neurons have been demonstrated to be highly dependent on olfactory bulb input for their survival – bulbectomy in adult rodents produces selective apoptosis of semilunar neurons (Friedman & Price, 1986). Here, we examined whether sensory deprivation alone, rather than complete physical removal of afferents, could enhance apoptosis of neurons in aPCX. Adult Long-Evans rats either had one naris sealed with cautery or served as cautery controls. After a survival period of 7 to 10 days, the brains were removed and processed for TUNEL reaction staining to label the apoptotic cells in the aPCX, and a blind cell count was done. Results indicated that for the sensory-deprived group, there was an approximately 50% increase in the number of apoptotic cells on the side ipsilateral to the closed naris compared to the contralateral hemisphere of the same animal and compared to control animals. These significant differences were due to increased cell counts in layer I/II; no difference between groups was apparent in layer III counts. These results suggest that short-term modulation of sensory experience can influence the survival of neurons in sensory cortex. Funded by NSF: IBN9808149 to DAW.

184 Poster Neural Plasticity and Regeneration

ABLATION OF BULB NEURONS KILLS PIRIFORM NEURONS BUT NOT SENSORY NEURONS

Guthrie K.¹, Gall C.² ¹*Biomedical Sciences, Florida Atlantic Univ., Boca Raton, FL;* ²*Anatomy and Neurobiology, Univ. of California, Irvine, CA*

Mature olfactory sensory neurons undergo apoptotic cell death following bulbectomy, suggesting that bulb neurons provide retrograde trophic cues necessary to their survival. However the resulting axotomy, rather than loss of specific trophic support, may trigger cell death. We compared the effects of bulbectomy with those produced by bulbar NMDA injection in rats. This lesion kills bulb neurons but leaves the sensory axons intact. We characterized the bulbar effects of NMDA treatment and examined the olfactory epithelium, bulb and piriform cortex for signs of cell death and atrophy after lesion. Studies combining Nissl staining, Fluorochrome labeling and TUNEL showed that after bulbectomy, cell death in ipsilateral epithelium and piriform cortex peaked at 32-48 hrs and 24 hrs, respectively. Though NMDA lesion killed most bulb neurons within 48 hrs, as late as 6 days postlesion the olfactory epithelium appeared normal and the incidence of cell death was similar to controls. However, cell death peaked in piriform cortex at 32-48 hrs postlesion. Ensheathing glia survived NMDA lesion, as evidenced by continued expression of NPY mRNA. Moreover, expression of OMP mRNA by sensory neurons was retained in the epithelium and outer bulb laminae up to 3 wks postlesion. The nerve layer remained intact and glomerular-like structures were evident as clusters of neuropil immunoreactive for OMP and GAP43. Reactive astrocytes were numerous. These data suggest that sensory neurons survive loss of putative trophic support from bulb neurons, while piriform neurons do not. Supported by grant DC03547 from NIDCD.

AXOTOMY VS. BULBECTOMY: TEMPORAL ANALYSIS OF APOPTOSISShinners M.J.¹, Robinson A.M.¹, Conley D.B.¹, Kern R.C.¹
¹Otolaryngology, Northwestern University, Chicago, IL

Olfactory receptor neurons (ORN) are bipolar sensory neurons that project axons to second order neurons in the antero-basal telencephalon (olfactory bulb). ORNs are unique in that they undergo a baseline rate of apoptosis, even in the absence of obvious disease, and are replaced by the mitosis and maturation of progenitor cells present in the epithelium. Regenerating neurons must synapse in the bulb, from which they obtain trophic factors necessary for long term survival. Studies have demonstrated that ORN apoptosis is dramatically upregulated following section of the axon (axotomy) or removal of the synaptic target (bulbectomy). Both techniques have been utilized as models of neural injury and apoptosis although the two have not been directly compared. The current study will track the rate of apoptosis as a function time following axotomy and bulbectomy in the BALB-c mouse using the TUNEL technique. Animals undergoing sham surgery serve as a control. Analysis was performed at 1.5, 4, 8 and 21 days following surgery. Results indicate that the rate of neuronal cell death as measured by TUNEL rapidly returns to control levels following axotomy. Animals undergoing permanent synaptic target removal (bulbectomy) demonstrate a chronically elevated rate of apoptosis. This study suggests that regenerating ORNs, following axotomy, are able to obtain trophic support from the bulb if left in situ. Following bulbectomy, regenerating neurons are fated to undergo apoptosis. Supported by Northwestern University Research Fund.

DOES ESTROGEN PROTECT OLFACTORY RECEPTOR NEURONS FROM APOPTOSIS?Pitovski D.Z.¹ ¹Smell and Taste Center, Dept of Otolaryngology, Wake Forest University, Winston-Salem, NC

Recent findings indicate that estrogen is neuroprotective, preventing neurons from entering the program cell death pathway (or apoptosis). In the olfactory neuroepithelium a significant proportion of neuronal loss occurs through the program cell death pathway. The cell death machinery, which is invoked in this process, consists of effectors, activators, and negative regulators including the product of bcl-2. New data suggests that bcl-2 may play a key role in olfactory neuronal survival. To begin evaluating the hypothesis that estrogen modulates both expression of bcl-2 and vulnerability of olfactory receptor neurons to apoptosis we examined olfactory levels of bcl-2 in the presence and absence of estrogen in Sprague-Dawley female rats. Furthermore, if a regulator/product relationship between estrogen and bcl-2 expression operates in olfactory receptor neurons, then one predicted functional consequences of this relationship would be relative protection against apoptosis. The results indicate that estrogen has a profound effect on olfactory bcl-2 expression and olfactory neuronal apoptosis occurs in estrogen-deprived animals.

APOPTOSIS OF OLFACTORY RECEPTOR NEURONS INDUCED BY INFECTION AND OLFACTORY NERVE TRANSECTION IN RATGe Y.¹, Tsukatani T.², Miwa T.² ¹ENT, Beijing University, Beijing, China; ²ENT, Kanazawa University Hospital, Kanazawa, Japan

Nasosinusitis and head injury are common causes for acquired hyposmia or anosmia. To investigate histochemical changes in olfactory receptor neurons under these conditions, we developed two animal models in rat, a sinusitis model and an olfactory nerve transection model. We used a primary antibody against PGP9.5 to show the normal olfactory receptor neurons and an antibody against single-strand DNA to show apoptotic cell death. In addition, Bcl-2 and Bax were also used to explore factors involved in the apoptosis of olfactory receptor neurons. In the sinusitis model a severe inflammatory reaction occurred on the experimental side, and nearly no inflammatory changes were detected on the control side. Apoptosis of olfactory receptor neurons was observed in both sides. However, apoptosis on the control side started later and behaved the similar time course as the infected side. Bcl-2 and Bax was detected only on the infected side. In the olfactory nerve transection model, significant neuron apoptosis was detected on the lesioned side only. No Bcl-2 or Bax positive cells were seen in the olfactory nerve transection model. According to this study, both olfactory nerve transection and sinusitis can cause olfactory receptor neuron apoptosis, but they appeared to act by different mechanisms. Apoptosis induced by unilateral sinusitis occurred not only on the infected side, but also on the control side where there was no evidence of local inflammation. The differences suggest different mechanisms of apoptosis. Bcl-2/Bax family seems to play an important role in the apoptosis induced by infection, but appeared to have no relationship with the effect on the control side or with nerve transection induced apoptosis.

GLUTAMATE RECEPTOR DISTRIBUTION IN THE OLFACTORY BULB IS ALTERED FOLLOWING NARIS OCCLUSIONHamilton K.A.¹, Coppola D.² ¹Cellular Biology and Anatomy, Louisiana State University Health Sciences Center, Shreveport, LA; ²Neuroscience Program, Centenary College, Shreveport, LA

The olfactory system appears to be uniquely suited for studies of glutamate receptor plasticity. Glutamate receptors are widely expressed by the olfactory bulb neurons, and the olfactory sensory neurons, which turn over throughout life, are glutamatergic. In other systems, changes in synaptic inputs alter expression of glutamate receptor subtypes, leading to changes in synaptic efficacy. Naris occlusion is known to alter the anatomy of the ipsilateral olfactory bulb and to result in decreased expression of the neurotransmitter, dopamine, apparently due to loss of the sensory inputs. We therefore postulated that naris occlusion might also result in altered glutamate receptor expression by olfactory bulb neurons. To test this hypothesis, the left or right naris of male and female mice was occluded on postnatal day (PD) 1, and the distribution of glutamate receptor subtypes was evaluated on PD 6, 12, 18, and 24 using well characterized antisera (Chemicon) and immunoperoxidase methods. Light microscopic examination on PD 6 and 12 failed to reveal any gross morphological differences or alterations in receptor distribution. As previously reported by others, however, by PD 18 and 24, the external plexiform layer (EPL) was narrower and the olfactory bulb was noticeably smaller on the occluded side. Moreover, with one antiserum, the glutamate receptor immunostaining pattern was strikingly altered in the EPL and in the adjacent mitral cell layer (MCL). We are currently quantifying differences between the occluded and non-occluded sides. Additional studies will be aimed at identifying the synaptic locations of glutamate receptor subtypes that exhibit altered expression and the mechanisms underlying the alterations.

DEVELOPMENTAL ACTIVATION OF EXTRACELLULAR SIGNAL-RELATED KINASE (ERK1/2) IN OLFACTORY BULB GRANULE CELLS IS ALTERED BY NARIS OCCLUSIONMirich J.M.¹, Brunjes P.C.¹ ¹Psychology, University of Virginia, Charlottesville, VA

Mitogen-activated protein kinase (MAPK) signal transduction pathways have been implicated in many fundamental cell processes including proliferation, growth, differentiation and responses to stress. A key kinase in the pathway, ERK1/2 (p44/42^{MAPK}), plays a central role in cell survival and synaptic plasticity. To assess developmental patterns and the role of synaptic activity on ERK1/2 activation, immunolocalization of the phosphorylated protein was examined in the olfactory bulbs of rats that had undergone unilateral naris closure on postnatal day (P) 1. Bulbs were examined at P10, 20, 30 or 40. In all tissue, approximately 90% of positively stained cells were located in the granule cell layer (GCL). Both control and experimental bulbs exhibited peak numbers of positive profiles at P20, however, many fewer profiles were seen in bulbs ipsilateral to occluded nares. Indeed, experimental bulbs had 50% fewer ERK1/2 (+) profiles at P10, although the difference decreased to 25% by P40. Approximately 25% (P10) to 33% (P20-40) of the profiles exhibited intense staining. The distribution of these darkly stained cells in the GCL changed as a function of age. By P20, 2/3 of the profiles were found in the superficial half of the layer in control bulbs. Occlusion skewed the distribution; 4/5 of the dark cells were found in the superficial GCL in P20 experimental bulbs. The results indicate that levels of afferent activity modulate ERK1/2 activation in granule cells, suggesting that the MAPK pathway is important in bulb development. Supported by HD-00338.

NGO & NGO RECEPTOR EXPRESSION IN THE MAMMALIAN OLFACTORY SYSTEMIwema C.L.¹, Bartolomei J.C.¹, Strittmatter S.M.², Greer C.A.¹¹Neurosurgery, Yale University School of Medicine, New Haven, CT;²Neurology, Yale University School of Medicine, New Haven, CT

The mammalian olfactory system is ideal for examining axon growth and correct targeting *in vivo* due to its regenerative capacity and the precision with which the olfactory sensory neurons (OSN) project to the olfactory bulb (OB). However, the cues that facilitate targeting and stabilization of OSN axons within the OB are not well understood. Recently, Nogo and its receptor (NgR) have been demonstrated to inhibit axon regeneration in the adult CNS. In support of this inhibitory role, experiments in which Nogo activity was neutralized with the IN-1 antibody following CNS injury resulted in axonal elongation and functional recovery. To determine if Nogo and NgR contribute to OSN axon growth/guidance in the mouse olfactory system, we used standard immunohistochemical procedures with antibodies to Nogo and NgR on olfactory tissue from P0 CD-1 mice. We find that Nogo is located along the entry zone of the outer olfactory nerve layer (ONL) and on mitral cells within the external plexiform layer. In addition, Nogo strongly co-localizes with laminin, a marker of olfactory ensheathing cells. NgR is observed in the projection neurons within the OB and also in the outer ONL but does not co-localize with laminin. Recent data from our lab demonstrated that the ONL is differentially organized; axon extension may occur primarily in the outer ONL whereas specific glomerular targeting may occur in the inner ONL. Thus Nogo and NgR may signal the growing OSN axons to stop at the outer ONL and prepare for sorting in the inner ONL. Supported by NIH: NS10174.

MASH1 AND NEUROD EXPRESSION IN METHYL BROMIDE-LESIONED ADULT RAT OLFACTORY EPITHELIUM.Manglapus G.L.¹, Youngentob S.L.¹, Schwob J.E.² ¹Dept. of Neuroscience and Physiology, SUNY Upstate Medical University, Syracuse, NY; ²Dept. Anatomy and Cellular Biology, Tufts University, School of Medicine, Boston, MA

Mash1 and *NeuroD* are basic helix-loop-helix (bHLH) transcription factors known to play a role in neurogenesis. In the olfactory epithelium (OE), *Mash1* and *NeuroD* are expressed by olfactory progenitor cells but their exact roles are incompletely understood. Based on a variety of data, it appears that *Mash1* is expressed by neuronally committed transit amplifying cells. *NeuroD* is expressed downstream of *Mash1* expression but at which precise stage of neurogenesis and its role is unclear. Methyl Bromide (MeBr) exposure destroys all cell types of the OE. Immediately post lesion, at least some globose basal cells (GBCs) are multipotent and can give rise to both neuronal and non-neuronal cells. Moreover, neurogenesis is suspended for a time. Therefore, assessing *Mash1* and *NeuroD* expression post MeBr may help elucidate their respective roles during olfactory neurogenesis. Using *in situ* hybridization along with a set of neuronal markers (GAP-43, TUJ-1, and NCAM) we examined *Mash1* and *NeuroD* expression following MeBr exposure to define the stages of GBC differentiation during reconstitution of MeBr-lesioned olfactory epithelium on a molecular level. Preliminary results indicate that both *Mash1* and *NeuroD* expression are increased post MeBr. However, unlike *Mash1* expression, *NeuroD* expression first undergoes a transient decline before being up-regulated, consistent with a role in the population of immediate neuronal precursors. Supported by NIH R01 DC02167 and NIH R03 DC04851-02.

IMMUNOSTAINING OF APOLIPOPROTEIN E IN MURINE MUCOSAKleene N.K.¹, Pixley S.K.¹ ¹Cell Biology, Neurobiology & Anatomy, University of Cincinnati, Cincinnati, OH

Olfactory receptor neurons (ORNs) regenerate throughout adulthood. The axons of newly formed ORNs elongate through an olfactory nerve that contains ensheathing cells and previously established axons. The ensheathing cells have unique neurite-promoting abilities. The factors responsible for the neurite-promoting abilities are not completely understood; however, one candidate is apolipoprotein E (apoE). ApoE is found in the olfactory nerve, and the most common human apoE isoform (apoE3) increases neurite outgrowth in other neurons. Nathan and coworkers (Experimental Neurology 172: 128-136, 2001) have shown that apoE levels increase in the olfactory bulb following lesioning of the mucosa in mice. In preparation for using the mouse to investigate the role of apoE in the olfactory mucosa, we immunostained adult murine nasal tissue sections with an antibody to apoE. ApoE labeling was very similar to that seen by Yamagishi and coworkers (Ann Otol Rhinol Laryngol 107:421, 1998) in the human mucosa. There was heavy labeling in nerve bundles and in blood vessel walls. In the epithelium, labeling was seen along the basement membrane, just apical to the basement membrane, and in the ORN-containing region. Tissues treated with normal goat serum or without primary antibody were not labeled. After double labeling with anti-ApoE and anti-S100, which labels ensheathing cells, some overlap was seen in the nerve bundles. This work is supported by the Univ. of Cincinnati, Dept. of Cell Biology, Neurobiology, and Anatomy.

CHEMOKINE-MEDIATED INFILTRATION OF MACROPHAGES INTO THE OLFACTORY EPITHELIUM FOLLOWING TARGET ABLATION

Getchell T.V.¹, Subhedar N.¹, Shah D.S.¹, Hackley G.¹, Partin J.V.¹, Sen G.¹, Getchell M.L.¹ ¹*Sanders Brown Ctr. on Aging, U. of Kentucky, Lexington, KY*

The C-C subfamily of chemokines are small bioactive proteins whose activities are mediated by binding to target cell receptors that belong to a family of G protein-coupled receptors. We investigated the expression of two C-C chemokines, macrophage inflammatory protein-1 (MIP-1) and monocyte chemoattractant protein (MCP-1), and their cognate receptors CCR1 and CCR2 respectively, in the murine olfactory epithelium (OE) following olfactory bulbectomy (OBX). Using ELISA, there was a transient upregulation of both chemokines from at or below the limit of detection (MIP-1, 1.5 pg/ml; MCP-1, 9 pg/ml); MIP-1 protein peaked at 4.4 ng/ml at 3 d post-OBX, and MCP-1 at 42 pg/ml at 16 hr post-OBX. Using relative quantitative RT-PCR, a transient upregulation of mRNAs for the chemokines and their receptors peaked at 2-3 d post-OBX. Digoxigenin-labeled MIP-1, MCP-1, CCR1 and CCR2 probes transcribed from RT-PCR products were synthesized for non-isotopic *in situ* hybridization. There was a systematic increase in the numbers of mRNA⁺ cells in the OE that peaked at 16 hr post-OBX; adjacent tissue sections stained with CD68 and F4/80 antibodies suggested that the mRNA⁺ cells were resident and infiltrating macrophages. Our studies indicate that the chemokines MIP-1 and MCP-1, signaling through CCR1 and CCR2 respectively, participate in intercellular signaling mechanisms by which the degeneration of olfactory neurons is coupled with phagocytosis and the proliferation of progenitor cells leading to neurogenesis and regeneration of the OE. Support: NIH-NIA R01 AG-16824-21 (TVG)

INFLAMMATORY CELLS IN THE NORMAL AND DENERVATED LINGUAL EPITHELIUM

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It is now acknowledged that the immune system is involved in the degeneration of peripheral nerves. Leukocytes—a general term for immune cells—are quickly recruited to sites of neural damage. In addition to their duties as phagocytes, leukocytes can affect neural and receptor cell function through cytokine secretion. They also migrate through tissues, giving them widespread access to target cells. Previous work showed that local communication can occur between intact and denervated taste receptor populations (Hill and Phillips, 1994), and leukocytes are attractive cellular mediators for this interaction. Moreover, systemic upregulation of immune function, which includes activation and proliferation of leukocytes, increases sodium taste responses after unilateral chorda tympani (CT) nerve sectioning (Phillips and Hill, 1996). In current work, it is proposed that leukocytes infiltrate the tongue after CT section, and serve as cellular messengers between receptor populations. Adult rats received unilateral CT sectioning, and frozen sections of tongue were stained with monoclonal antibodies R73 and V65 to identify T cells, or ED1 to identify macrophages. Leukocytes were identified, counted, and localized with respect to taste buds in sections of tongue. Preliminary evidence shows that macrophages and T cells are present in normal fungiform papillae and surrounding epithelium, and that their numbers increase after chorda tympani nerve sectioning. These data support a novel role for the immune system in neural and receptor cell degeneration in the taste system.

TIME-COURSE OF TASTE BUD REGENERATION AFTER TRANSECTION OF THE GLOSSOPHARYNGEAL AND GREATER SUPERFICIAL PETROSAL NERVES IN THE RAT

St. John S.J.¹, Garcea M.², Spector A.C.² ¹*Department of Psychology, Reed College, Portland, OR;* ²*Department of Psychology, University of Florida, Gainesville, FL*

Taste buds degenerate when the nerve supply is interrupted and reappear following reinnervation. We have previously reported anterior tongue taste bud regeneration following chorda tympani nerve transection in the middle ear reaches an asymptote of ~70% at 42 days (d). Here we determined the time course of taste bud regeneration, a proxy for nerve reinnervation, after transection of the glossopharyngeal (GL) and greater superficial petrosal (GSP) nerves. The GL was transected (GLX) close to the medial external wall of the bulla in rats that were perfused 14, 28, 42, 56, or 70 d after surgery (n=6/group). The GSP was transected (GSPX) in the middle ear and rats were perfused at 14, 28, 42, 56, 112, or 224 d after surgery (n=4-5/group). The circumvallate papilla (CV), the soft palate (SP), and the nasoincisor ducts (NID) were excised, embedded, sectioned, and stained with hematoxylin and eosin. After GLX, taste buds reappeared in the CV in a linear fashion starting between 15 and 28 d and at 70 d reached 80.3% the number found in sham operated controls (n=12). Less than 25% of the control (n=7) number of taste buds were seen collectively in the SP and NID 112 d after GSPX and no further increase was seen at 224d, suggesting that the GSP does not substantially regenerate following transection in the middle ear. These experiments provide useful parametric data for investigators studying the behavioral consequences of gustatory denervation and functional competence of the regenerated peripheral gustatory system. Supported by R01-DC01628.

RECOVERY OF SALT TASTE RESPONSES AFTER CRUSH OF THE CHORDA TYMPANI NERVE IN MICE.

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Recovery of salt responses and its amiloride (Ami) sensitivity after crush (AC) of mouse chorda tympani (CT) nerve was studied by examining responses of single fibers and whole nerve of the regenerated CT and behavioral discrimination between NaCl and KCl. At about 2 weeks AC-CT, behavioral discrimination between NaCl and KCl disappeared and no significant responses of the nerve to taste stimuli were observed. At about 3 weeks AC-CT, responses to salts recovered in both behavioral and neural measurements. However, at this period the behavioral salts discrimination was not evident and NaCl responses of the CT were not significantly inhibited by Ami. Almost all single fibers responded to NaCl were also sensitive to KCl, but were insensitive to Ami (E-type). At about 4 weeks AC-CT, mice became behaviorally discriminative between NaCl and KCl, and showed small but significant Ami inhibition of CT responses to NaCl. Also, some single fibers best responding to NaCl and showing the Ami inhibition (N-type) reappeared. After more than 5 weeks, mice showed complete recovery in Ami sensitivity in neural responses and the behavioral salts discrimination. These results suggest that Ami-insensitive taste cells innervated by E-type fibers reappeared earlier than Ami-sensitive cells innervated by N-type. Reappearance of Ami-sensitive N-type fibers AC-CT play a crucial role on recovery of behavioral discrimination between sodium and potassium salts.

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DEVELOPMENTAL TASTE RECEPTOR CELL KINETICSHendricks S.J.¹, Brunjes P.C.¹, Hill D.L.¹ ¹*Psychology, University of Virginia, Charlottesville, VA*

A dynamic relationship exists between receptor cells and innervating neurons in the rat gustatory system. The size of mature taste buds is directly related to the number of innervating neurons. Moreover, fungiform taste buds increase in volume during development, a function that can be accurately predicted based on the number of innervating neurons at postnatal day 10. At least one component of the volume increase during development is addition of taste receptor cells. This growth function is retarded in animals raised on a low sodium diet from conception, preventing the match between taste bud volume and innervation number from occurring. To identify kinetic changes in taste receptor cell production and lifespan during normal and sodium-restricted development, tritiated thymidine injections were administered to neonate and adult sodium-replete rats, and to adult rats raised on a low sodium (0.03% NaCl) diet from conception. Labeled cells within taste buds were counted and average receptor cell lifespan was determined to be the length of time required to reach a half maximal rate after peak labeling within the taste bud. Animals aged ten days at the time of thymidine injection show nearly identical lifespan calculations as compared to sodium replete adults (i.e., approx. 10 days). However, basal cells appear to be more mitotically active in young rats, producing more cells in the same amount of time. In contrast, sodium-restricted adults show profound alterations in taste receptor cell kinetics. Supported by NIH grants P01 DC00407 and HD07232

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SEMA3A REPELS LATE EMBRYONIC STAGE SENSORY AXONS THAT PENETRATE SEMA3A MRNA-RICH LINGUAL EPITHELIUM.Dillon T.E.¹, Rochlin M.W.¹ ¹*Biology, Loyola University (Chicago), Chicago, IL*

Fungiform papillae on the anterior rat tongue are innervated by trigeminal and geniculate ganglion axons. We previously showed that E12-13 pre-tongue explants repel axon outgrowth from these ganglia, and that Semaphorin 3A (Sema3A) signaling is necessary and sufficient for this repulsion (e.g., Rochlin et al., J. Comp. Neurol. 422:579-593). Despite a decrease in long range repulsion by parasagittal tongue explants at E14 and later stages, Sema3A mRNA persists throughout the dorsal epithelium through E18. Since axons begin to penetrate the fungiform papilla epithelium at E17 (Farbman & Mbiene, *ibid.* 306:172-186), it is important to determine what role, if any, Sema3A plays. We are first testing the hypothesis that loss of Sema3A sensitivity enables these afferents to enter the epithelium. Cell line explants secreting Sema3A repelled E18 trigeminal axons in collagen gel co-cultures. The strength of repulsion depended on the growth factor used to promote outgrowth. The tendency of E18 geniculate ganglia to dissociate *in vitro* hindered analysis of repulsion. However, a semi-quantitative analysis of the axon outgrowth revealed a significant repressive influence of Sema3A on geniculate axon outgrowth. Thus, *in vitro*, sensory axons are still sensitive to Sema3A when they would be penetrating the Sema3A-mRNA rich epithelium *in vivo*. We have also begun to test whether the epithelium of dorsal tongue explants repels sensory ganglion axons at close range after the decline in long range repulsion. Preliminary observations suggest that axons long enough to contact the epithelium tend not to do so. NIH Grant R03 DC04965-01A1.

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SHH PROTEIN IN EMBRYONIC RAT TONGUE AND TONGUE CULTURESLiu H.¹, Maccallum D.K.², Gaffield W.³, Mistretta C.M.¹ ¹*School of Dentistry, University of Michigan, Ann Arbor, MI; 2Medical School, University of Michigan, Ann Arbor, MI; 3Regional Research Center, USDA, Albany, CA*

Identification of mRNA for Sonic hedgehog (Shh) protein in taste papillae of embryonic rodent suggests a regulatory role in papilla development. However localization of Shh protein has not been described. We previously used a steroidal alkaloid, cyclopamine, and a blocking antibody to Shh, to disrupt Shh signaling in embryonic whole tongue cultures, and demonstrated papilla- and stage- specific roles for Shh. At embryonic day (E) 13 in rat, fungiform papillae were increased in size but not number when Shh signaling was perturbed, whereas at E14 papillae were doubled in number and located on posterior as well as anterior tongue. By E16, disrupting Shh had no effect on papilla patterning. To further understand the role of Shh in papilla development, we have used a rabbit polyclonal antibody to Shh and immunohistochemistry to locate Shh in rat embryo tongues from E13-18, and in tongue cultures at E13 and 14. In E13 tongue Shh is diffuse in lingual swellings, but by E14 broad, irregular patches of Shh are on anterior tongue only. At E16-18, Shh is intense in each fungiform, and the circumvallate papilla. In tongue cultures with standard or cyclopamine medium, Shh is intensely immunolocalized in each fungiform papilla, even those that form in large numbers on posterior tongue with cyclopamine. Shh is very dense in the basement membrane region of the apical papilla epithelium, and some protein is in nearby mesenchymal cells. Our demonstration that Shh is in new fungiform papillae that develop in culture after signal disruption, suggests that Shh is a taste papilla marker and a true morphogen in papilla development. Supported by NIH NIDCD Grant DC00456 to CMM.

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RETINOIC ACID ACTS DIRECTLY TO RESPECIFY OROPHARYNGEAL EPITHELIUM.Rougas S.¹, Parker M.A.¹, Barlow L.A.² ¹*Department of Biological Sciences, University of Denver, Denver, CO; 2Department of Cell and Structural Biology, University of Colorado Health Sciences Center, Denver, CO*

During development, taste buds arise directly from oropharyngeal epithelium. In amphibians, the ability of the epithelium to generate taste buds is independent of nerve or mesenchymal cell contact, and is acquired very early. During gastrulation, the notochord signals to presumptive pharyngeal endoderm, so that by the end of gastrulation, the endoderm is specified to make taste buds (Barlow, 2001). To explore this process further, we asked if specification of pharyngeal endoderm was reversible. Retinoic acid (RA) is known to cause loss of anterior head structures when gastrula to neurula stage embryos are exposed to this teratogen. In our hands, exposure of axolotl early neurulae to 10-5M RA resulted in larvae with truncated head structures, including a reduced pharyngeal volume and loss of most taste buds. However, the effect of RA on these endodermal derivatives may have been indirect, through its well-documented action on adjacent ectoderm and mesoderm. To test this, we removed pharyngeal endoderm from neurulae, then exposed it to RA, and compared taste bud numbers with those of control explants. Taste buds were virtually abolished in RA-treated explants, indicating that the teratogen acted directly on the endoderm. Further, although pharyngeal endoderm is specified to make taste buds during gastrulation, these new data show that this tissue remains plastic until later neurula stages, and thus can be respecified. We are currently examining the cell and axial fates of pharyngeal endoderm exposed to RA. Supported by NIDCD, DC03128 & DC03947.

DIFFERENTIAL EFFECTS OF GUSTATORY NERVE TRANSECTION ON QUININE-STIMULATED FOS-LIKE IMMUNOREACTIVITY IN THE PARABRACHIAL NUCLEUS OF THE RAT

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Intraoral infusion of 0.003 M quinine hydrochloride stimulates a population of Fos-like immunoreactive (FLI) neurons within specific regions of the gustatory portion of the nucleus of the solitary tract. This population of quinine-stimulated FLI-neurons is severely attenuated following bilateral transection of the glossopharyngeal nerve (GLX) but not the chorda tympani nerve (CTX; King et al., 1999). For the current study, we are re-examining the brains of these same animals to assess the effects of these nerve cuts on the numbers of FLI-neurons within specific subdivisions of the parabrachial nucleus (PBN) that have been implicated in gustatory processing. Data from the subjects currently analyzed confirm our earlier report that GLX (N=3) attenuates the number of quinine-stimulated FLI-neurons in the "waist" region of the PBN to a level comparable to water-stimulated SHAM rats (N=3) while having no effect on the number of quinine-stimulated FLI-neurons in either the external lateral or external medial subdivisions. Bilateral CTX (N=3) apparently has little effect on the number of quinine-stimulated FLI-neurons within any of these PBN subdivisions. These data compare favorably with behavioral data demonstrating that the number of gapes, a stereotypical oromotor rejection response, is reduced substantially more by bilateral GLX than by CTX (Travers et al., 1987; Grill et al., 1992). Supported by NIDCD R01-DC01628

DENATONIUM, PROPYLTHIOURACIL AND QUININE ELICIT SIMILAR PATTERNS OF FOS-LIKE IMMUNOREACTIVITY IN THE RAT NUCLEUS OF THE SOLITARY TRACT

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Molecular studies demonstrate a large number of G-protein receptors for bitter stimuli, with mRNAs for multiple receptors expressed on single taste receptor cells (Adler et al., 2000; Chandrashekar et al., 2000). However, Ca⁺⁺ imaging suggests that individual receptor cells discriminate between these chemicals (Caicedo & Roper, 2001). We used Fos immunohistochemistry to assess whether 3 stimuli that rats reject and humans rate as bitter, evoked differentiable patterns of Fos-like immunoreactivity (FLI) in the 1st-order taste relay, the rostral nucleus of the solitary tract (rNST). Rats (n=8) were implanted with intraoral cannulae, adapted to fluid stimulation (7ml water/30 min) in daily sessions, then received either water, 3mM QHCl, 10mM propylthiouracil, 2.7mM denatonium, or 30mM citric acid. At 45 minutes post-stimulation, rats were anesthetized then perfused, and frozen brainstem sections processed using ABC/DAB techniques. Each tastant elicited at least 2X as much FLI in the rNST as water. The 3 bitter stimuli elicited highly similar topographic patterns of FLI (all r's > .85), clustered in the medial NST. This pattern contrasted with the more lateral FLI after acid (all r's between acid and bitter stimuli < .2). These results suggest that various bitter tastants activate common or co-mingled neurons in a circumscribed NST region, but cannot rule out a differential topographic activation of cells that do not express FLI, or neurons at higher levels of the neuraxis. Supported by DC00416 & the OSU NGSP

SIGNAL TRANSDUCTION PATHWAYS IN BITTER TASTE DETECTION

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Many bitter stimuli are believed to bind to specific G-protein-coupled membrane receptors on taste cells. Biochemical studies suggest that bitter taste receptors are coupled to G-proteins that activate either a phospholipase C (PLC) or a phosphodiesterase (PDE) signaling pathway, or both. Several components of these pathways (G-proteins and downstream effectors) have been identified in taste buds but how they play a role in bitter transduction requires functional validation. To study the cellular and molecular mechanisms of bitter taste, we have recorded activation of taste cells in lingual slices (Ca²⁺ imaging) and identified intracellular signaling components in the same cells (immunohistochemistry). In mice vallate papillae, 45% (19/42) of bitter-responsive cells were immunostained for alpha-gustducin. In agreement with this correlation, the incidence of cells responding to bitter stimuli (cycloheximide, quinine, and denatonium) was reduced by more than half in mutant mice lacking gustducin. However, some cells in gustducin knock-out mice were sensitive to bitter stimuli, and even in wild type mice, many bitter-responsive cells were not immunostained for gustducin. We are currently testing whether the signaling components of the PLC pathway are also associated with bitter responses. We are correlating responses to cycloheximide, denatonium, or quinine with the expression of PLC-beta2 and other downstream effectors. Supported by NIH/NIDCD grants DC00374 (S.D. Roper) and DC04525 (A.C.).

PHYSIOLOGICAL EXPLORATION OF BITTER AND SWEET RECEPTORS IN RAT TASTE RECEPTOR CELLS.

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Recent molecular studies suggest classes of bitter (e.g. T2Rs) and sweet (e.g. T1Rs) receptors are segregated across taste receptor cells (TRCs) whereas physiological studies suggest TRCs may be broadly tuned to these stimuli. Using patch clamp analysis on posterior TRCs, we examined responses to cycloheximide (CHX) and saccharin (SAC). When inhibition of outward potassium currents was measured (typically 15-30%), 42% of cells responded to 100 μ M CHX (n=64 of 151), 44% responded to 20 mM SAC (n=93 of 211) and 24% of cells responded to both (n=23 of 94). Denatonium (100 μ M; DEN) was also tested and inhibited 61% of tested cells (n=48 of 79). The number of cells responding to both CHX and SAC is much larger than predicted by molecular expression studies. BAPTA (a calcium chelator) and bisindolylmaleimide (BIS, a PKC inhibitor) were used to test for underlying transduction mechanisms. BAPTA was without effect on SAC responses (n=9) but increased the mean inhibition of CHX by 10% (n=14). BIS treatment demonstrated that some CHX and SAC responsive cells are PKC sensitive whereas others are insensitive. Effects on inwardly rectifying potassium current (Kir) was also examined. SAC increased Kir in 21% of cells (10 of 47), CHX increased in 13% (8 of 59), and denatonium (DEN) decreased in 37% of cells (9 of 24). Additionally DEN strongly inhibited sodium currents, which was not observed with other stimuli. These results suggest that sweet and bitter responses may involve multiple receptors and transduction mechanisms that may be more complicated than predicted by a simple examination of receptor expression pattern across TRCs. Supported by NIH DC00401 and NSF IBN9724062

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Bitter Taste

SOA & CAFFEINE FORM NON-NORMAL TASTE DISTRIBUTIONS UNRELATED TO PROP/PTCBreslin P.A.¹, Tharp C.D.¹, Reed D.R.² ¹*Sensory, Monell Chemical Senses Center, Philadelphia, PA;* ²*Genetics, Monell Chemical Senses Center, Philadelphia, PA*

As part of a large project to determine individual sensitivities to several bitter tasting compounds, we have observed frequent insensitivities to sucrose octaacetate (SOA [2×10^{-4} M]) and caffeine [2.1×10^{-2} M]. Subjects rated these two solutions on a Labeled Magnitude Scale in the context of rating eight bitter compounds including PROP. Insensitivity to SOA and caffeine occurred in approximately 30% of the sample population and formed non-normal frequency distributions. The study of SOA sensitivity has the advantage of examining a human phenotype that parallels the well-characterized mouse SOA taste insensitivity. The short-term goals of this study are to carefully characterize the frequency of SOA/caffeine insensitivity, to describe the phenotype in detail, and to determine the heritability and genetic transmission of SOA and caffeine insensitivity. The long-term goal is to identify the genes responsible for these specific bitterness insensitivities via a linkage analysis approach. This work was supported by NIH 02995 to PASB.

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Bitter Taste

TASTE INTERACTIONS AMONG BINARY MIXTURES OF BITTER COMPOUNDSBournazel M.M.¹, Keast R.S.², Breslin P.A.² ¹*Taste, Firmenich SA, Geneva, Switzerland;* ²*Taste, Monell Chemical Senses Center, Philadelphia, PA*

Recent advances in the bitter taste literature indicate many receptors and multiple pathways for bitter taste transduction. Interactions between similar tasting compounds can result in synergy, suppression, or additivity. Such results give us insight into transduction mechanisms involved with the individual compounds. In this study we investigated interactions among eight bitter compounds: tetralone (iso-alpha acid mixture), denatonium benzoate, sucrose octaacetate, quinine-HCl, ranitidine, L-tryptophan, L-phenylalanine, and urea. Psychometric curves were constructed for all compounds for each subject (n=17) from bitterness ratings produced on the Labeled Magnitude Scale. From the psychometric curve of each bitter compound, four concentrations were chosen from the linear phase and combined sequentially with a single weak concentration from each of the other seven compounds. A weak concentration of sucrose was used as a control-additive. Interactions were compound specific and resulted in examples of synergy, suppression, and additivity. Supported by NIH grant DC02995 to PASB

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Bitter Taste

CAPSAICIN AS A GUSTATORY STIMULUSGreen B.¹, Schullery M.¹ ¹*Sensory Neuroscience Group, John B. Pierce Laboratory, New Haven, CT*

Capsaicin is considered a prototypical 'trigeminal' stimulus. However, a preliminary study of the sensitivity of the back of the tongue indicated capsaicin can evoke a bitter taste on the circumvallate (CV) papillae. Two experiments confirmed this observation: In exp. 1, 25 Ss rated the taste and irritation of 100 uM capsaicin, 0.5M sucrose, 0.5M NaCl, 0.025M citric acid, and 1.0mM QSO4 applied to the CV region with cotton swabs. 15 Ss who reported '>weak' bitterness from capsaicin on the LMS were tested further to determine the intensity of capsaicin bitterness and burning on three tongue sites: the tip, the side (near the foliate papillae), and the CV papillae. Menthol (100 mM) was also tested for comparison with capsaicin. Both irritants evoked significant bitterness, especially in the CV region, where capsaicin's bitterness was judged 4 times stronger than on the tongue tip. In contrast, burning from capsaicin was stronger on the tip than in the CV region. To rule out the possibility that bitter ratings resulted from response bias rather than from a true taste, 16 new Ss (screened as before from a total of 31) were tested on the tongue tip and CV region with capsaicin plus 0.5M sucrose (which should inhibit bitterness), 1.0mM QSO4 (which should enhance bitterness), or dH2O. Adding QSO4 significantly increased bitterness ratings, whereas sucrose suppressed bitterness in the CV region by 80% relative to capsaicin in dH2O. These results imply capsaicin and menthol stimulate gustatory fibers that respond to bitter tasting substances. The regional and individual differences further suggest that these fibers are more common in cn. IX than cn. VII, and that their incidence varies across people. Supported in part by NIH grant DC05002.

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Response Properties of Central Olfactory Neurons

DYNAMIC GATING OF SPIKE PROPAGATION IN THE MITRAL CELL SECONDARY DENDRITES FOR INTERACTIONS WITH DIFFERENT COMBINATION OF OLFACTORY GLOMERULIXiong W.¹, Chen W.R.¹ ¹*Neurobiology, Yale University, New Haven, CT*

One unique feature of the olfactory bulb circuits is the mitral cell lateral secondary dendrites. Through their long projection (1000-2500 μ m) and numerous dendrodendritic reciprocal synapses, these dendrites link one olfactory glomerulus to roughly 300 out of a total of 2000 glomeruli; but the functional principles governing this extensive lateral interaction remain largely unknown. Here we report that fast Na^+ action potentials were able to propagate actively through the entire length of secondary dendrites. However, the actual extent of propagation was regulated dynamically by local inhibitory synaptic inputs distributed along the dendrites. The extent of propagation in turn determined the spatial pattern of Ca^{2+} influx in these presynaptic dendrites and thus the range and number of dendrodendritic synapses to be activated. Accordingly, network control of spike traffic in the mitral cell secondary dendrites can contribute to dynamic coupling and uncoupling of widely distributed olfactory glomeruli for the processing of different odorants. This work was supported by grants to W.R.C. from the National Institute on Deafness and Other Communication Disorders (R01-DC03918) and from the Whitehall Foundation (#9814).

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ODORANT INDUCED EXPRESSION OF ARC MRNA IN MOUSE MOB PERIGLOMERULAR CELLS

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Activity-regulated cytoskeleton-associated protein (ARC) is expressed in rat periglomerular cells (PG) in response to odorant stimulation (Guthrie et al, 2000), resulting in glomerular patterns similar to those identified by cFOS and 2-DG labeling. Since olfactory receptor neurons (ORNs) expressing a given receptor project to one or two glomeruli in the lateral and medial regions of the MOB, the set of glomeruli activated by a particular odorant likely reflects the olfactory receptors (ORs) that bind to that odorant. Mapping the co-distribution of glomeruli associated with odorant elicited ARC-labeled PG cells and glomeruli receiving projections from individual ORs will identify a set of odorants that bind to individual ORs. Digoxigenin-labeled ARC cRNA probes were in situ hybridized (ISH) to MOB sections from C57BL/6 mice exposed for 30 min to a series of odorants. PG labeling was most extensive in the caudal-lateral and caudal-medial MOB in response to hexanone, 2-pentanone, butyraldehyde, caproaldehyde, valeric acid, butyl acetate and hexyl acetate. The distributions of glomeruli associated with labeled PG cells were similar to those of glomeruli labeled by 2-DG methods after equivalent odorant stimulation. 33-P-labeled olfactory receptor (OR) cRNA probes, derived from ORs PCR-amplified from glomerular tissue punches taken from the caudal-lateral MOB (Yang and Marchand, 2002), were hybridized to MOB sections, resulting in one or two labeled glomeruli in the caudal-lateral and caudal-medial MOB. Double-label ISH procedures are currently being developed to identify individual glomeruli activated by odorants and receiving input from individual ORs, to characterize the molecular range of odorants binding to individual ORs.

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ADAPTOR PROTEINS MODULATE PROTEIN-PROTEIN INTERACTIONS AND BIOPHYSICAL PROPERTIES OF AN OLFACTORY BULB K⁺ CHANNEL

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Two adaptor proteins expressed in the olfactory bulb differentially modulate v-Src-induced Kv1.3 (*Shaker* family K⁺ channel) phosphorylation and modulation of channel function via alteration of SH2- and SH3-mediated protein-protein interactions. Grb10 adaptor significantly reduces v-Src-induced Kv1.3 phosphorylation, whereas tyrosine phosphorylation of Kv1.3 is modestly increased in the presence of n-Shc adaptor. The proline rich sequences contained in Grb10 adaptor protein may compete for the SH3 domain of Src, to decrease the ability for Src to phosphorylate Kv1.3 and suppress Kv1.3 current magnitude, increase the inactivation time constant (τ_{inact}), and disrupt cumulative inactivation during repetitive voltage stimulation. N-Shc adaptor also acts to prevent the v-Src-induced increase in the Kv1.3 τ_{inact} but additionally reverses a shift in voltage-dependence typically observed for Kv1.3 in the presence of v-Src. Through site-directed mutagenesis of the regulatory Tyr residues in the CH domain of n-Shc and comparison of the activity of n-Shc with a close family member Sck, data suggest that a portion of the CH domain that includes tyr^{221,222 and 304} and is homologous to Sck regulates a shift in Kv1.3 voltage-dependence and inactivation kinetics. Collectively these data indicate that the repertoire of adaptor molecules expressed in olfactory bulb or other neurons and the specific capacity for regulating the proximity of kinase to its effector (the ion channel) may influence the electrical phenotype of a neuron via phosphorylation. Supported by NIH R29DC03387.

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GLOMERULAR, BEHAVIORAL AND BIOPHYSICAL CHANGES IN THE OLFACTORY BULB OF KV1.3 KNOCK-OUT MICE.

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The voltage-gated potassium channel, Kv1.3, carries a large proportion of outward current in olfactory bulb neurons (OBNs) and can be modulated by several tyrosine kinases including the insulin receptor. Mice with gene-targeted deletion of Kv1.3 (KO) were examined to understand the channel's contribution to olfactory bulb (OB) function. In simple behavioral experiments, the retrieval time to uncover litter-hidden food items as opposed to marbles is significantly shorter in the KO animals. Using the Metamorph program, image analysis of cresyl violet- and DAPI-stained cryosections indicate that KO mice have a greater number of glomeruli that are individually smaller than those of WT mice. Initial counts of neuronal versus glial cell-types in the OB show no loss in population numbers, implying that the cells may simply be re-distributed. Electrophysiologically, the OBNs cultured from KO mice have slow inactivation kinetics and lack cumulative C-type inactivation, which is a hallmark biophysical property of Kv1.3 current. The neurons are not modulated by acute application of insulin, although insulin receptor expression is equivalent in WT and KO animals. Deletion of the Kv1.3 channel also increases expression of the TrkB and src kinases, a finding that is consistent with work by others using heterologous expression systems. In summary, loss of Kv1.3 channel alters OBN current, expression of modulatory kinases, glomerular units, and gross olfactory detection. Supported by NIH R29DC03387 and RO1DC01919.

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RESPONSES OF OLFACTORY BULB UNITS IN THE CHANNEL CATFISH TO AMINO ACID ISOMERS

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A recent calcium imaging study of neuronal activity of olfactory receptor axonal terminals in the zebrafish olfactory bulb (OB) reported that the L-conformation of an amino acid (AA) is an absolute requirement for activation of AA-responsive olfactory receptors (Fuss & Korsching, 2001). Previous behavioral experiments indicated, however, that channel catfish could discriminate among various AA isomers (Valenticic and Caprio, unpublished). Presently investigated was the excitatory response properties of single OB neurons to AA stereoisomers. Thirty-eight units located within the AA-responsive region of the catfish OB (Nikonov and Caprio, 2001) at a depth of 200-300 μ m were each tested with L & D-isomers of methionine (Met), alanine (Ala), arginine (Arg) and glutamate (Glu). All 38 units tested were spontaneously active (5.6 ± 2.3 spikes/s) and each unit responded excitedly to only one of the AAs [11 Met, 8 Ala, 13 Arg and 6 Glu units]. Thirty-four units responded solely to a specific L-isomer at 1 μ M & 10 μ M, but some also responded weakly to the 100 μ M D-isomer; synchrony between the simultaneously recorded local field potentials and spikes occurred for all responses but the latter. Four units [1 Met, 2 Arg, 1 Glu] responded to the D-isomer, and 2 [1 Met, 1 Arg] of these responded specifically to the D-AA. Estimated electrophysiological threshold for the L-AA-responsive units was 0.1 μ M, whereas that for the few D-AA-responsive units was 1-10 μ M. The present results suggest that OB units in channel catfish can show high specificity to amino acids, and that only small numbers of these units are responsive to D-AAs. Supported by NIH DC-03792

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MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS DISTINGUISH FOUR CLASSES OF JUXTAGLOMERULAR NEURONS IN THE MAIN OLFACTORY BULB

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Juxtaglomerular cells (JG) of glomerular layer in the main olfactory bulb (MOB) comprise the first neuronal ensemble involved in odor analysis. From whole cell recordings of physiologically characterized and biocytin-filled/reconstructed JG cells in the rat MOB slices, four morphologically separable JG cell types have been identified: (1) Short-axon (SA, n=11), classical periglomerular (CPG, n=22), hairy periglomerular (HPG, n=17) and external tufted cells (ET, n=31). HPG and CPG cells have significantly smaller soma area and total dendritic length than ET cells; SA cells are intermediate. Three physiological parameters differed significantly ($p < 0.05$) among cell types: (1) Input resistance (MOhm) was greatest in HPG (1458 ± 185), intermediate in SA (642 ± 114) and CPG (866 ± 114) and lowest in ET (441 ± 104). (2) Fast afterhyperpolarizations (mV) in SA (20.8 ± 1.3) and CPG (14.7 ± 1.5) were deeper than in HPG (4.8 ± 1.9) and ET (6.3 ± 1.4). (3) All ET and most (73%) CPG cells receive monosynaptic ON input; all HPG cells and most (66%) SA cells receive polysynaptic ON input. Spontaneous generation of spike bursts was typical for ET cells (67%), while generation of plateau-potentials was observed in most (70%) HPG and some (32%) CPG cells. Taken together, these findings indicate that juxtaglomerular neurons fall into at least 4 distinct classes. PHS grants: DC03195, DC02588, DC00347 & NS36940.

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CRAYFISH PARASOL CELLS EXHIBIT MULTIPLE BURST INITIATION SITES

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Parasol cells are multimodal sensory interneurons in the crayfish lateral forebrain, characterized by extensively branched dendritic arbors within the hemi-ellipsoid body. They are targets of projection neurons from the accessory lobes, which transmit indirect olfactory, visual and tactile input via axons in the olfactory-globular tract. Parasol cells exhibit coherent, periodic EPSPs and accompanying single spikes, which arise from autoactive neural centers within the eyecup. Strong sensory input evokes impulse bursts in parasol cells, at the peaks of the periodic activity. We currently do not know where bursts arise within a parasol cell, but assume they are generated by driver potentials that are confined to restricted regions of the neuron. We now report evidence that each parasol cell has multiple sites for the initiation of impulse bursts. 1) Sharp electrode recordings from sites in the major dendritic branches often exhibit not only invading impulse bursts but one or more classes of electrotonically degraded bursts. 2) While local, invasive bursts transiently suppress background depolarizing activity and accompanying local impulses, distantly recorded bursts have no effect upon local spiking but do suppress distantly recorded single spikes. Furthermore, local, invasive bursts have little or no effect upon the frequency of distantly recorded spikes due to background activity. These data suggest that major branches of the dendritic arbor have independent burst initiating zones and, furthermore, that the transient depressive effects of each of these multiple burst initiation zones are confined to that portion of the dendritic tree throughout which the burst can actively propagate.

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ULTRA-LONG-LASTING DEPOLARIZATIONS IN RESPONSE TO OLFACTORY NERVE INPUT IN DEVELOPING MITRAL CELLS.

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The mouse main olfactory bulb (MOB) undergoes extensive post-natal development, with mitral cells (MCs), the principal cells of the MOB, showing remarkable dendritic reorganization. At post-natal day 0 (P0) MCs have multiple apical dendrites entering multiple nascent glomeruli, but no lateral dendrites. By P8 they have a single apical dendrite forming a tuft within a single glomerulus, and multiple lateral dendrites. We have investigated MCs using whole-cell recording in mouse brain slices, before, during and after postnatal dendritic reorganization. MC resistance falls from ~ 1 Gohm at P0 to adult levels (~ 200 MOhm) by P15, while membrane potential increases from -40 mV to -55 mV. Adult MCs produce a long-lasting (~ 500 msec)^{*,*} depolarization (LLD) in response to ON stimulation. Early post-natal MCs produce a LLD of remarkable duration: LLDs increase from ~ 1 s at P0 to a maximum of 15 s by P4, falling to adult durations by P8. As in adult MCs, early post-natal LLDs are abolished by AMPA/Kainate receptor blockade, but not NMDA receptor blockade. The decrease in membrane resistance is consistent with the outgrowth of mitral cell lateral dendrites, and increased membrane surface area. LLD duration is maximal at the time of apical tuft formation and retraction of supernumerary apical dendrites, falling as MC morphology and local circuit organization matures. Studies are underway to investigate the relationship between LLD duration and the dendritic organization of individual MCs, at different developmental stages. Support: DC00347.

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BACK-PROPAGATING ACTION POTENTIALS ACTIVATE DIFFERENTIAL SPATIAL CALCIUM RESPONSES IN MITRAL CELLS IN RAT OLFACTORY BULB SLICES

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The aim of this study was to compare back-propagating action potential (BAP) activated Ca^{2+} responses in mitral cell soma, primary dendritic trunk and tuft. The study was carried out on rat olfactory bulb slices; methods were based on custom-made two-photon microscopy and patch clamp techniques, measuring fluorescence with Oregon Green. BAP elicited the smallest Ca^{2+} increase in mitral cell somas (F/F, $6.9\% \pm 1.1$). The Ca^{2+} increases in proximal, middle and distal dendritic trunk were approximately 21.0%, 27.6% and 21.3%, respectively, with no significant difference (student t test). The increases in the proximal tuft (from branching level I to IV, starting from the origin of the tuft) were approximately 45.0%, 39.2%, 52.0% and 43.7%, respectively, with no significant differences. Significant differences were present between soma and primary dendritic trunk ($P < 0.0005$), between primary dendritic trunk and the proximal glomerular tuft branching levels I-IV ($P < 0.01$), and between tuft levels I-IV and more distal tuft branches ($21.1\% \pm 3.0$) ($P < 0.005$). These results suggest that the rat mitral cell has at least four Ca^{2+} compartments: soma, primary dendrite trunk, proximal glomerular branches (I to IV) and distal glomerular branches ($> IV$). We speculate Ca^{2+} fluxes play different roles in each of these compartments in controlling transmitter release, membrane excitability through Ca^{2+} -activated K^+ channels, and neuronal plasticity. Supported by NIDCD, Human Brain Project and MURI grant.

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MODELING BISTABILITY AND RESONANCE IN THE MEMBRANE DYNAMICS OF MITRAL CELLS.

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Recent experimental studies of intrinsic properties of rat main olfactory bulb mitral cells (MCs) have revealed their bistable dynamics. MC membrane voltage has two stable equilibria separated by about 10 mV: a relatively depolarized up-state in which voltage oscillations and action potentials occur, and a down-state in which action potentials do not occur. MC membrane potential can be switched between the down state and the up state by synaptic input from the olfactory nerve (ON), and from the up-state to the down-state by a transient hyperpolarizing input. In the up-state, MCs are more likely to generate an action potential following ON input. We have developed a simple Morris-Lecar style conductance-based model that qualitatively reproduces MC bistable behavior. The model consists of fast persistent Na⁺ current with wide activation range, a slower persistent K⁺ current with a narrow activation range, and an Ohmic leak current. It is described by three ordinary Hodgkin-Huxley type differential equations. The simple dynamic model predicts that in the down-state the neuron acts as an integrator, while in the up-state the neuron acts as a resonator, with sustained oscillations. We are continuing to develop a detailed conductance based model, based on MC voltage clamp recordings. Supported by DC00347, DC02173 & DC36940 (MTS).

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MEMBRANE BISTABILITY AND SUB-THRESHOLD OSCILLATIONS IN AN OLFACTORY BULB MITRAL CELL MODEL

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In a recent study (Heyward et al, J. Neurosci, 21:5311-5320; 2001) mitral cells were found to be bistable, exhibiting two preferred membrane-potential states separated by 10 mV. Sub-threshold membrane potentials were seen in the upstate, and have also been observed in other studies. It was hypothesized that the bistability was produced by the interaction between a non-inactivating sodium current and a slowly inactivating potassium current. It has also been hypothesized that the sub-threshold oscillations depend upon a non-inactivating sodium current. We have developed computational models of the mitral cell to test these hypotheses and explore the functional consequences of the bistable and oscillatory behaviors. Our models give excellent qualitative and good quantitative agreement with the results of Heyward et al, and are able to reproduce all the principal features of the mitral cell activity. Analysis of the conditions required for bistability and/or oscillations allow us to understand how ion channel properties affect the behavior and predict how bistability/oscillations may be enhanced or suppressed. We also explore the consequences of bistability/oscillations for the information processing capabilities of the mitral cell. This work was supported by NIH grants DC-00086 and DC52552 (Human Brain Project), and a Multiuniversity Research Initiative (DOD).

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FLAVOR EFFECTS ON PERCEIVED TEXTURE AND FOOD INTAKE

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Previously, it was demonstrated that added flavors affected specific oral texture attributes of vanilla custard desserts, namely creamy mouthfeel, and rough and fatty afterfeel (de Wijk et al., AChemS 2001). Added flavors also resulted in increased prickling. These results were replicated in a follow-up study where benzaldehyde (almond flavor) added to vanilla custard desserts affected the same four oral attributes. The use of nose clips, which eliminated the influence of olfaction and nasal irritation on perceived texture, eliminated the effects of benzaldehyde on these attributes. These results demonstrate the influence of nasal stimulation on specific oral attributes. It is unclear whether these effects were caused by olfaction or by nasal irritation. Differences in preferred bite size between vanilla custard desserts disappeared when benzaldehyde was added demonstrating the role of olfaction in food intake. Differences in preferred bite size between custard desserts increased when nasal stimulation was eliminated (with nose clips) demonstrating that food texture, and possibly taste, is also important for food intake.

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BITTERNESS AND ASSOCIATED ORAL SENSATIONS IN CHLORHEXIDINE/ETHANOL RINSES

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Chlorhexidine digluconate (CHLX) is one of the most effective antigingival/antibacterial agents used in therapeutic oral care products. In oral rinses, CHLX is in solution with ethanol which creates interesting formulation challenges, since CHLX and alcohol are both bitter and impart moderate 'oral sensations'. The aim of the present study is to determine the interactions among the oral qualities that CHLX and ethanol share in common. Two concentrations of CHLX (0.038% and 0.12% w/v) were mixed with two concentrations of ethanol (3% and 10% w/v) and assessed by 21 trained subjects using a Labeled Magnitude Scale (LMS) over a 5-minute period following tasting. 'Oral sensations' co-occurring with bitterness were 'tingling', 'warm/hot' and 'numbing'. The mixture of ethanol and CHLX increased the intensity of both the bitterness and the 'oral sensations' above that elicited by CHLX alone, especially when mixed with 10% w/v ethanol. Bitterness and 'oral sensations' were dose-dependent. The intensities of bitterness from CHLX and alcohol were enhanced when mixed together, but did not combine additively. At the levels tested, the oral qualities of CHLX and ethanol combined sub-additively. But in a more diluted rinse, which should have better sensory properties, their combined effects may be different than observed here.

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EFFECTS OF CHLORHEXIDINE ON THE TASTE OF CHLORIDE SALTSKolesar M.R.¹, Gent J.F.¹, Frank M.E.¹ ¹*Oral Diagnosis, University of Connecticut Health Center, Farmington, CT*

Perception of the saltiness of all salts tested and the bitterness of a subset of the salts (Breslin and Tharp, 2001; Frank *et al.*, 2001) are reduced after rinsing with chlorhexidine gluconate (CX), a bitter, bisbiguanide antiseptic. CX dose dependence was measured by having 12 human subjects rate the intensity and identify the quality of 100mM NaCl, NH₄Cl, KCl and CaCl₂, 1mM quinine-HCl (QHCl) and water before and after 3, 1-min rinses with 15 mL of CX (0.1, 0.3, 1.0mM) or 1mM QHCl. All subjects participated in 4 sessions; in each session, two replicates of the 6 test stimuli were rated before and at 5 and 20 min after a treatment. Repeated measures ANOVAs were used to analyze data. The subjects rated the bitter 1mM QHCl and 1mM CX treatment rinses equally intense. Treatment with 0.3mM and 1mM CX reduced the intensity of monovalent salts by 34% ($p < 0.003$) and 51% ($p < 0.0001$), respectively; however, neither 0.1mM CX nor 1mM QHCl had any effect. No treatment affected the intensity of CaCl₂ or water. "Salty" responses decreased for all salty stimuli (NaCl, KCl, NH₄Cl, and CaCl₂) ($p < 0.0001$): "salty" response frequencies for these stimuli were 71% after water, 72% after QHCl, and 54%, 42%, and 35% after 0.1mM, 0.3mM, and 1mM CX rinses, respectively. Of the bitter stimuli (QHCl, CaCl₂, KCl, NH₄Cl), "bitter" responses were reduced only for QHCl ($p < 0.05$), being 93% after water, 94% after QHCl, but 56% after 1mM CX. The results show that chlorhexidine affects taste in a dose-dependent way that does not depend on its bitterness *per se*, and provide additional support for a unitary salty percept. Supported by NIH grant P50 DC00168.

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GUSTATORY-IRRITANT INTERACTIONS: SUPPRESSION OF TASTE BY ORAL CAPSAICINBoucher Y.M.¹, Simons C.T.², Carstens E.¹ ¹*Neurobiology, Physiology & Behavior, Univ. of California, Davis, Davis, CA*; ²*Food Science & Technology, Univ. of California, Davis, Davis, CA*

Capsaicin induces oral irritation by exciting trigeminal endings, and is reported to reduce the intensity of certain tastes by an unknown mechanism. We tested if oral capsaicin affects the responses of gustatory neurons in the solitary nucleus (NTS), the first central relay of the taste pathway. In pentobarbital-anesthetized rats, single-unit recordings were made from gustatory NTS neurons identified by their response to one or more of the following tastants applied to the anterior tongue: 0.03 M citric acid, 0.1 M NaCl, 0.3 M sucrose, 0.2 M monosodium glutamate (MSG) and 0.003 M quinine. The magnitude of response elicited by the most effective tastant was recorded before and again after constant-flow application of capsaicin (33 μ M) to the tongue for 7 min. Some gustatory NTS neurons were excited and some inhibited, while most (75%) did not respond during application of capsaicin. After capsaicin there was a significant attenuation of NTS unit responses to citric acid (to 50%), sucrose (63%), NaCl (44%), and MSG (73%). Quinine-evoked responses were also depressed >50% in the few units tested. Thirty min after capsaicin, sucrose, NaCl- and citric acid-evoked responses had fully recovered, indicating that capsaicin desensitization has no lasting effect on taste transmission, while MSG-evoked responses were still partly depressed. These data show that pretreating the lingual epithelium with capsaicin suppresses tastant-evoked neural activity. Whether these interactions occur peripherally or centrally is currently under study.

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NICOTINE SUPPRESSION OF TASTANT-EVOKED NEURAL ACTIVITY IN THE RATSimons C.T.¹, Boucher Y.M.², Carstens E.² ¹*Food Science & Technology, Univ. of California, Davis, Davis, CA*; ²*Neurobiology, Physiology and Behavior, University of California, Davis, Davis, CA*

Reduced taste intensity may contribute to the anorectic effect of tobacco. We investigated if nicotine, an irritant in tobacco, affects neural taste transmission. Recordings were made from single gustatory neurons in the nucleus of the solitary tract (NTS) of barbiturate-anesthetized rats. Each unit's response to the most effective of 5 tastants (NaCl, citric acid, sucrose, glutamate, quinine) was recorded prior to and following pretreatment of the tongue with nicotine (0.87, 8.7, 600 mM) for 4 min. NTS units were excited by nicotine alone at the 2 higher concentrations. Interestingly, nicotine excitation of NTS units was reduced by the nicotinic antagonist, mecamylamine. Following cessation of nicotine application, averaged tastant-evoked responses were significantly reduced in a manner related to nicotine concentration (to 54 and 20% at 8.7 and 600 mM nicotine, respectively). Responses recovered to pre-nicotine levels within approximately 9 min. Nicotine suppressed NTS units' responses to each of the 5 tastants tested. These data indicate that nicotine, an irritant that tastes bitter, may excite gustatory neurons via a nicotinic receptor-mediated mechanism. Furthermore, nicotine has a strong depressant effect on tastant-evoked activation of NTS neurons via mechanisms that are currently under investigation.

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SUCROSE-DENATONIUM AND DULCIN-QUININE ANTAGONISM IN NEURAL RESPONSES TO TASTE MIXTURESFormaker B.K.¹, Hettinger T.P.¹, Frank M.E.¹ ¹*Oral Diagnosis, University of Connecticut Health Center, Farmington, CT*

When presented as a taste mixture, quinine hydrochloride (QHCl) suppresses responses to sucrose in the hamster (*Mesocricetus auratus*) chorda tympani nerve (CT) (Formaker & Frank, 1996; Formaker *et al.*, 1997). Other combinations of "bitter" and "sweet" stimuli might show a similar suppression. To test this hypothesis, whole-nerve responses to mixtures of sucrose and denatonium benzoate (DB) and mixtures of dulcin and QHCl were measured. DB and QHCl behaviorally cross-generalize in hamsters, as do sucrose and dulcin; all four compounds are effective CT stimuli (Bouwer *et al.*, 1997; MacKinnon *et al.*, 1999). Binary mixtures were prepared so concentrations in mixtures equaled component concentrations. Combinations of 0, 30, 100 and 300 mM sucrose with 0, 3, 10 and 30 mM DB; and combinations of 0, 1, 3 and 10 mM dulcin with 0, 1, 3 and 10 mM QHCl were used. Solutions containing 10 mM dulcin were prepared just before use to avoid precipitation. Like sucrose-QHCl, the sucrose-DB and dulcin-QHCl mixtures showed significant suppression of 5-10 sec steady-state CT responses. Subtraction of responses to the QHCl or DB component revealed mixture suppression that increased with QHCl or DB concentration. At 10 mM QHCl and 30 mM DB, suppression was at least 70%. Although QHCl and DB may simply be acting as modulating drugs, the results are consistent with a mechanism that involves depletion of cAMP by QHCl and DB in sucrose-responsive taste receptor cells, and suggests an antagonism between bitter and sweet taste stimuli. Supported by NIH grant DC 04099.

ROLE OF PRIOR ASSOCIATIONS IN THE SUB-THRESHOLD INTEGRATION OF TASTES AND ODORS

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Sub-threshold integration of a congruous odor-taste pair, but not an incongruous pair (Dalton et al., 2000), showed that the central neural integration of multi-modal inputs might contribute to our perception of flavor. It remains unclear why the incongruous pair of stimuli failed to integrate. To determine whether prior pairings and associations play a role in the summation of tastes and odors, we tested for sub-threshold integration of otherwise incongruous pairs of stimuli prior to and following a three-week exposure in the form of flavored chewing gum. Following exposure, the combined detection threshold for the pair decreased, demonstrating newly acquired central integration, while the combined thresholds of exposed but unpaired odors and tastes remained stable. Therefore, the failure of "incongruous" tastes and odors to integrate may be attributed to a lack of prior paired associations. This work was supported by NIH Grant DC02995 to PASB and DC03704 to PD.

ANALYSIS OF MULTICOMPONENT ODOR MIXTURES BY HONEYBEES

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Natural odor signals are most often composed of mixtures of chemicals of a variety of molecular structures. Furthermore, odor mixtures can vary both qualitatively and quantitatively as animals experience different odor objects from the same reward class (e.g., flowers that present nectar to a honeybee forager). The primary focus of our research was to investigate how such variability affected the perception of mixtures and their components. Specifically, we examined four parameters: quantitative variation in each component of a mixture; the mean mixture osmolality; the molecular similarity of chemical components; and the coefficient of variation for all the odor mixtures experienced during learning. We trained honeybees to 3-component odor mixtures over 16 trials in a way that produced robust conditioned responding. We then evaluated generalization from the mixture to individual components. All four parameters have an effect on recognition of the individual components of a blend. For example, as the geometric mean of the osmolality rises, the probability of the response to low level components decreases, which is perhaps mediated by gain control in the olfactory system. In addition, when the odor components are dissimilar in molecular composition, they are more likely to elicit a response if they are present at high, constant concentrations in the mixture. These experiments demonstrate that both qualitative and quantitative variation in multicomponent odor mixtures affect what information animals are able to extract from the mixture.

ASYMMETRIC INTERACTIONS BETWEEN HETEROGENEOUS RETRONASAL AND ORTHONASAL ODORANT PAIRS

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Twenty subjects (median age 20, 11 female) each selected concentrations of anise, cinnamon, coffee, peppermint, and strawberry food-grade liquid extracts for which vapor phase orthonasal (ortho), or retronasal (retro), intensities matched ortho or retro vapor phase intensity of 33% diluted orange extract, and then learned retro, and ortho, veridical names of the matched concentrations and of the orange standard. In a later session, after retro and ortho identifications (ID) of the 6 odorants, 10 randomized ortho & retro pairs of odorants were given 3 times (9 heterogeneous pairs; one homogeneous), with instructions to either initially exhale (retro 1st) or inhale (ortho 1st), and to report ID(s) and their order, if more than one odor smelled. Odorant presentation containers were used (Pierce and Halpern, *Chem.Senses*, 21, 529-543, 1996). RESULTS: For heterogeneously paired odorants, the frequencies of correct IDs of the odorants in retronasal locations, measured independently of the order in which they were reported, were equivalent to IDs when the odorants were presented retronasal-only, but for the majority of heterogeneous pairs, IDs of the odorants in the orthonasal location were significantly less correct than IDs of the same odorants when presented only in an orthonasal location. When order of reported IDs for a pair was considered, IDs in which not only correct odorant names but also correct order of smelling was preserved were significantly less common than IDs for which the order of smelling was reversed. B.C. Sun support: NY Sci. Ed. Prog.

SPECIFICITY OF SUCROSE-BEST FIBERS IN RHESUS MONKEY CHORDA TYMPANI DOES NOT DEPEND ON STIMULUS CONCENTRATION

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Previously we demonstrated that information from only sucrose-best (S) fibers in chorda tympani (CT) is sufficient to separate sweeteners from compounds of other taste qualities. The purpose of this study was to test whether the S as well as other fibers maintain their specificity when stimulated with higher concentrations. Method: Recordings were obtained in 8 rhesus monkeys during stimulation of the tongue with 4 different concentrations of NaCl, citric and ascorbic acids, quinine (QHCl), denatonium, sucrose and SC45647. Results: Using multi-unit spike separation technique we isolated 143 CT fibers. In agreement with our previous observations the S fibers were most narrowly tuned. Furthermore, the specificity of all S fibers did not change during stimulation with any concentrations of stimuli belonging to other taste qualities. Thus 19 out of 27 S fibers responded only to the sweeteners and did not respond to any concentration of NaCl, acids and QHCl and denatonium. Even in the remaining 8 S fibers, the responses to non-sweet stimuli were smaller than those to sucrose or SC45647. Among 52 QHCl-best fibers, 25 responded only to the bitter stimuli. In 27 QHCl-best fibers, however, acids at high concentrations also elicited strong responses. Some NaCl-best (15%) and acid-best (17%) fibers maintained their specificity at all concentrations of all stimuli used. Conclusion The S fibers of rhesus CT remain narrowly tuned in spite of changing stimulus concentrations. This suggests that S fibers play a determining role in coding of the sweet taste.

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Neural Coding and Circuits

ROLE OF AMINO ACID NEUROTRANSMITTERS IN TASTE-MOTOR PROCESSING

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Reversible lesions in the reticular formation (RF) ventral to the rostral nucleus of the solitary tract (rNST) suppress ingestion and rejection responses elicited by gustatory stimuli (Chen et al, *AJP*, 280:1085-1094, '01). The role of excitatory and inhibitory amino acids in taste-motor processing within this substrate was explored by infusing small volumes of neurotransmitter agonists or antagonists in a chronic, awake rat preparation and recording EMG responses evoked by gustatory stimulation. Bilateral infusions of the NMDA receptor antagonist DCP (0.198-1.98 nmol/100 nl) suppressed oral responses in a step-like (on-off) fashion, suggesting a role in response initiation. The neural substrate appears under tonic inhibition because infusions of the GABA_A antagonist bicuculline (0.026-0.125 nmol/60-100 nl) generated larger amplitude EMG responses. In contrast, infusions of the glycine receptor antagonist strychnine (6.74 nmol/100-200 nl) disrupted phase relations between muscle antagonists suggesting a role in phasic (reciprocal) inhibition. Differential effects on ingestion and rejection were not observed to the various pharmacological treatments. However, the amplitude and phase changes observed to the various drug manipulations mimicked motor aspects of the transition from licking in response to a preferred stimulus, to gaping in response to an aversive stimulus. It is postulated that the RF substrate ventral to the rNST is essential for producing this sensorimotor switch. Supported by DC00416 & DC00417.

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PARALLEL MAPPING OF MULTIPLE STIMULUS FEATURES USING MULTICHANNEL RECORDING ARRAYS IN THE MOTH ANTENNAL LOBE

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Optical imaging studies suggest that different odorants are spatially represented by the activity of distinct combinations of olfactory glomeruli, but there is disagreement over how specific features of the stimulus (e.g., different concentrations or blends of odorants) may be represented. This has led to speculation that temporal coding mechanisms may also underlie these spatial patterns. To examine the interactions between the spatial and temporal components of odor-evoked representations in the moth antennal lobe (AL), we are using a silicon-based microelectrode array that permits simultaneous recording (with high spatial and temporal resolution) from up to 16 sites across several glomeruli in the AL. In more than 80% of the preparations tested with general odorants, the patterns of ensemble activity evoked by qualitatively different odorants showed considerable overlap across multiple glomeruli, suggesting that the spatial pattern of activation alone was not sufficient for fine discrimination. Cross-correlation analysis of the same ensembles, however, revealed that each odorant synchronized a different subset of glomerular projection neurons within the coding ensemble. The subset of neurons that synchronized was also dependent on the concentration of the stimulus, and the ensemble of neurons synchronized by a blend of odorants was not a simple sum of the subsets synchronized by the individual odorants. Our results support the hypothesis that selective synchronization encodes stimulus context, and these temporal patterns are superimposed on the spatial pattern of glomeruli activated by a given odorant. Supported by a grant from NIH-NIDCD (DC02751).

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HIGHLY SPECIFIC OLFACTORY RECEPTOR NEURONS SPECIFIC TO NATURALLY PRODUCED ODOR LIGANDS IN DROSOPHILA MELANOGASTER

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Due to its well defined genome the fruitfly *D. melanogaster* has become a very important model organism in olfactory research. The first insect olfactory receptors were recently defined in this species. Despite all the research invested, few natural odor ligands have been identified. By using a combined gas chromatographic – single receptor neuron recording technique (GC-SC) we set out to identify active odor molecules in head space-collected volatiles from preferred food sources, i.e. different overripe or rotting fruit. In all we performed 101 GC-SC experiments on 87 contacted sensilla. In most recordings a single GC peak would produce a strong response, whereas a few other compounds would produce weaker responses. Using GC-massspectrometry we identified 29 active compounds. Our GC-SC recordings revealed that the olfactory receptor neurons (ORN) investigated could be divided into distinct functional types with discrete characteristics. The response patterns from individual neurons were repeatable and neurons were found to reside in stereotyped pairs. In total, we identified 9 physiologically distinct sensillum types based on the response profiles of their ORNs. Presently we are investigating the behavioral significance of the odors detected. This research project was funded by the Swedish Research Council

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RHYTHMIC BURSTING AND SYNAPTIC INTERACTIONS AMONG JUXTAGLOMERULAR (JG) NEURONS MAY TRANSFORM OLFACTORY NERVE (ON) INPUTS INTO SYNCHRONOUS ALL-OR-NONE GLOMERULAR OUTPUT.

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Glomeruli are targeted by olfactory receptor neurons with the same odorant specificity. Little is known about JG neurons or glomerular functions. Spontaneous activity and synaptic interactions among morphologically-defined JG neurons were determined by whole-cell recordings in rat bulb slices. External tufted cells (ET) exhibit rhythmic spike bursts (mean: 3.5 bursts/s) that persist in blockers of fast synaptic transmission, but are abolished by sodium channel blockers. They respond to ON stimulation with a constant latency EPSC, indicative of monosynaptic input. Short axons (SA) and many periglomerular (PG) cells exhibit spontaneous bursts of EPSCs. ON stimulation evokes longer, variable latency EPSC bursts; suggesting that these SA and many PG cells are polysynaptically linked to the ON, via ET and/or M/T cells. Pairs of JG cells in the same, but not different, glomeruli have synchronous events (membrane oscillations, spikes bursts, EPSCs) (correlation coefficient ~0.49). Direct synaptic coupling was only observed from ET to PG/SA cells, which appear to receive convergent input from multiple ET cells. A single ON shock causes a synchronous burst in all ET cells. This is followed by a prolonged period (~1 sec) of oscillatory bursting that parallels the time course of synchronous long-lasting depolarizations in mitral cells associated with the same glomerulus. The glomerular network thus may transform ON inputs to a synchronous, all-or-none mitral cell output. PHS grants: DC03195, DC02588, DC00347 & NS36940.

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SYNTHETIC CODING OF ODORANT MIXTURES IN RAT PIRIFORM CORTEX.

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Current views of odorant discrimination by the mammalian olfactory system include molecular feature extraction by the olfactory receptor sheet, feature amplification and contrast enhancement by the olfactory bulb, and feature synthesis into odorant perceptual wholes by the piriform cortex. Given the enormous number of odorant feature combinations possible in nature, however, it seems unlikely that cortical synthetic receptive fields (RF's) are innate, but rather require experience for their formation. The present experiment addressed two issues. First, we made a direct comparison of mitral/tufted cell and aPCX neuron abilities to discriminate odorant mixtures from their components to further test whether aPCX neurons can treat collections of features different than the features themselves (synthetic coding). Second, we attempted to determine the minimum amount of experience necessary for formation of cortical synthetic RF's. Single-unit recordings were made from mitral/tufted cells and aPCX layer II/III neurons. Cross-habituation between binary mixtures and their components were used to determine odor discrimination abilities. Preliminary results suggest that after at least 50 sec of experience with a binary mixture, aPCX neurons can discriminate the mixture from its components while mitral/tufted cells cannot. However, when limited to 10-15 sec of experience with the mixture, aPCX neurons appear similar to mitral/tufted cells and do not discriminate mixtures from components. These results suggest experience-dependent synthetic processing in aPCX, and suggest an important role for perceptual learning in normal odor discrimination. Funded by DC03906.

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LANDMINE DETECTION USING AN ARTIFICIAL OLFACTORY SYSTEM

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We are developing a portable, artificial olfactory system (the Tufts Medical School Nose, or TMSN) to detect buried landmines. In a manner based on dog behavior, the TMSN actively samples air from near the ground via sniffing to detect trace amounts of landmine-related compounds. Air samples are drawn over an array of diverse chemical sensors, which are used to detect and discriminate odors in the environment (see White & Kauer, 2001, AChemS XXIII). To test TMSN sensitivity, dilute vapors from landmines, explosives, and other compounds were presented to the device using an air-dilution olfactometer (calibrated by GC/Mass. Spec.) connected to a chamber normally used to train and test dogs. The TMSN showed 100% detection and no false alarms for the landmine-related compound DNT at concentrations down to approx. 500 pp-trillion, levels comparable to thresholds of the best dogs tested in the same chamber. To determine whether this sensitivity is sufficient for landmine detection, field tests were conducted at the DARPA landmine facility at Ft. Leonard Wood, MO. Sniffs over buried PMA1A anti-personnel landmines produced sensor signals associated with the mines. The TMSN was then tested on nine "blind" sites where the presence or absence of a PMA1A mine was unknown to the user. Mine detection was 100% with a false alarm rate of 40%. Although additional field tests are clearly needed, these preliminary data suggest that a device based directly on olfactory principles can detect the vapor signatures of buried landmines. Supported by DARPA, ONR, and NIDCD.

235 Symposium

Chemosensation: Psychophysical Measurement in the 21st Century**INTRODUCTION - CHEMOSENSATION: PSYCHOPHYSICAL MEASUREMENT IN THE 21ST CENTURY**

Marks L.E.¹ ¹*Epidemiology and Public Health/Psychology, Yale University, New Haven, CT*

Psychophysical scaling is concerned with the measurement, or quantification, of sensory and perceptual magnitudes. A variety of methods has been devised over the years to scale the magnitudes of sensations, in the chemical senses and elsewhere. The symposium addresses two main questions: First, what are the strengths and limitations of some of the important methods commonly used in scaling, such as magnitude estimation and category rating? And second, how have different approaches, such as absolute magnitude estimation, labeled magnitude scaling, functional measurement, and conjoint scaling, sought to validate sensory scales. The symposium will consider such matters as the nature of the assumptions that underlie different approaches to scaling, the consistency and reliability of results obtained through different methods, and the sensitivity of different methods to intergroup and interindividual differences.

236 Symposium

Chemosensation: Psychophysical Measurement in the 21st Century**ABSOLUTE MAGNITUDE ESTIMATION**

Gescheider G.A.¹ ¹*Psychology, Hamilton College, Clinton, NY*

Absolute magnitude estimation (AME) requires observers to match their subjective impression of the size of a number to their subjective impression of a stimulus, and to do so independently of prior matches. The method, developed by Zwisllocki and his associates, is based on the hypothesis that, at a particular time and setting, an observer assigns a number to a stimulus in such a way that the psychological magnitude of the number matches that of the stimulus. Forcing an observer to use an arbitrary number associated with a particular standard stimulus can, by violating the natural tendency to match psychological magnitudes, cause bias. Thus, observers are capable of making unbiased numerical judgements of the sensory magnitudes of stimuli only when permitted to use their own natural numbers. AME scales are absolute because they have the mathematical property of having a fixed unit and their validity is supported by tests of their additivity and transitivity. As with all psychophysical scaling methods, in AME, the judgment by the observer of the sensory magnitude of a stimulus has been found to be influenced by the context within which the stimulus is presented. For example, if a stimulus is presented after the presentation of several weaker stimuli, it is often judged to be more intense than when it is presented after the presentation of several stronger stimuli. Such a context effect can result from response bias or from perceptual contrast in which a stimulus is perceived to be more intense when presented within the context of weak than within the context of strong stimuli.

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THE PERILS OF ACROSS-GROUP COMPARISONS
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Direct comparisons of sensory or hedonic perceived intensities across individuals are impossible since we cannot share experiences. However, with some assumptions, we can make comparisons across groups. For example, individuals are born into one of three groups based on the bitterness of PROP (6-n-propylthiouracil). Bitterness can be assessed using a sensory standard (ie, magnitude matching; Marks et al, 1988); subjects rate tastes and sounds on a common intensity scale. Assuming no relation between taste and audition, we can compare average taste intensities across nontasters, medium tasters and supertasters of PROP. Remembered sensations (eg, brightness of the sun) can act as standards as well. Intensity adjectives/adverbs have also been used as standards (eg, Does this taste very strong?). But there is peril in doing this. Consider a vivid example from S.S. Stevens: a large mouse running up the trunk of a small elephant. Clearly a scale labeled small and large would be much smaller for mice than for elephants. Similarly, a labeled scale for sensory intensity can be compressed or stretched to fit a particular domain. The Labeled Magnitude Scale developed for oral sensations (Green, et al, 1993) represents the first time that intensity labels have been empirically placed on a ratio scale with the location of the maximum also noted. Since the maximum oral sensation is different across nontasters, medium tasters and supertasters, we generalized the LMS (gLMS) and placed "strongest imaginable sensation of any kind" at the top. Doing this produced similar separations of PROP groups using magnitude matching (sound standard) and the gLMS. Note: a sensory or remembered sensation standard can be added to the gLMS to combine these methods. (DC 00283)

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USING FUNCTIONAL MEASUREMENT TO STUDY CHEMOSENSORY RESPONSES

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Over the past several decades philosophers and historians of science have come to recognize that theory and "facts" (i.e., data) are inextricably linked. Theory defines what counts as data and observations ostensibly serve as the basis for verification, modification or rejection of theoretical tenets. Measurement bridges the gap between theory and data by specifying the procedures that may be legitimately used to collect data. A good example of the relationship between theory, measurement and data can be taken from astronomy's approach to measuring distance. The methods used to estimate distances to celestial bodies are grounded on basic theoretical assumptions about the nature of the solar system and universe. Norman Anderson (1992) advocates a similar approach to measurement in the psychological context. His functional measurement approach embeds scaling within the context of psychological theory. Although the approach is fundamentally psychological rather than psychophysical, it can be used to address issues in the psychophysical domain. Unlike some approaches to psychophysical scaling, functional measurement assumes that context and multiple causation are fundamental psychological phenomena and therefore should be incorporated into one's measurement scheme. Functional measurement emphasizes an empirical approach to scale validation, the importance of an explicit theoretical framework and the role of psychological variables in experimental outcomes. Studies of chemosensory mixtures are used to demonstrate the utility of the functional measurement approach as well as its limitations.

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NONMETRIC SCALING OF SENSORY EVENTS: ADVANTAGES AND DISADVANTAGES

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To construct interval or ratio scales of sensory magnitude using direct scaling techniques, one must assume that the sensory estimates generated by subjects are either linearly or proportionally related to sensory magnitude. In many instances (e.g., when testing special populations such as young children) assumptions of linearity or proportionality may be unwarranted. In contrast, nonmetric scaling techniques only require that subjects can consistently judge which of two sensations or which of two sensory intervals is larger. Thus, nonmetric techniques have a much broader range of applicability than standard scaling techniques. However, this advantage is offset by two factors: (1) nonmetric techniques typically require the collection of a significantly larger data set than do direct scaling techniques, and (2) the relationship between the measures generated by subjects and the resultant sensory scale is less transparent for nonmetric than for direct scaling techniques. In this presentation we describe the application of nonmetric scaling techniques to: (1) comparisons of sensory intervals between stimuli; and (2) comparisons of the conjoint effects of two or more factors on sensory magnitude. We will show that these nonmetric techniques are especially useful when there is some question concerning: (1) the validity of the linear or proportional relationship between judgments and sensory magnitude, (2) the dimensionality of the subjective experience, and/or (3) the additivity of the contribution of two factors to a sensory experience.

240 Poster Taste Receptor Cells

DROSOPHILA OBPS EXPRESSED IN TASTE ORGANS

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We identified a large family of putative odorant-binding protein (OBP) genes in the genome of *Drosophila melanogaster*. Some of these genes are present in large clusters in the genome. Most members are expressed in various taste organs, including gustatory sensilla in the labellum, the pharyngeal labral sense organ, dorsal and ventral cibarial organs, as well as taste bristles located on the wings and tarsi. Some of the gustatory OBPs are expressed exclusively in taste organs, but most are expressed in both olfactory and gustatory sensilla. Multiple binding proteins can be co-expressed in the same gustatory sensillum. Cells in the tarsi that express OBPs are required for normal chemosensation mediated through the leg, as ablation of these cells dramatically reduces the sensitivity of the proboscis extension reflex to sucrose. Finally, we show that OBP genes expressed in the pharyngeal taste sensilla are still expressed in the *poxneuro* genetic background while OBPs expressed in the labellum are not. These findings support a broad role for members of the OBP family in gustation and olfaction and suggest that *poxneuro* is required for cell fate determination of labellar but not pharyngeal taste organs.

CALCIUM PUMP ISOFORMS IN CHEMORESPONSE

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Paramecia are attracted to stimuli such as biotin, folate, acetate, cAMP, and ammonium through 3 or more pathways, at least two of which appear to involve the plasma membrane calcium pump (PMCA) for sustained hyperpolarization of the cell. In our study of the pumps, we find that there are at least 4 PMCA genes in *Paramecium*, 3 of which are expressed. Interestingly, cells expressing GFP-isoform-2 show defects in chemoresponse behavior while those expressing HA-isoform-3 show normal responses. However, both show evidence of defective pump activity and high intracellular calcium, implying that isoform 2 is the pump involved in chemoresponse transduction. Activation of calcium pumps occurs through phosphorylation, calmodulin binding to, or dimerization at the C terminal calmodulin binding domains (CBDs). We created mutants of the CBD of isoform 2 (CBD-2), with alanines or glutamates substituted for the serines of the putative phosphorylation site. Wild type CBD-2 binds calmodulin in overlays and is phosphorylated in vitro by PKA, PKC and CamKinase. Mutant CBD-2s are not phosphorylated, implying that the two serines that we mutated are the primary phosphorylation sites. CBD-3 and -4 are substrates for PKA and PKC but not CamKinase, implying some differentiation of regulation despite co-localization (see additional poster). All of the wild type and mutant CBD2-GST fusion proteins inhibit plasma membrane Ca-ATPase activity in vitro. We are exploring the NMR-structures of the CBDs to determine why this is so. Supported by DC 00721, GM59988 and the VCCC.

IDENTIFICATION OF RAT TASTE CELL TYPES EXPRESSING IP3R3

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Rat taste buds contain several types of taste cells distinguishable by morphological characteristics, however, the physiological roles of these cell types is not clear. IP₃ is an important second messenger in both bitter and sweet taste transduction, and we have shown previously that the type III IP₃ receptor (IP₃R3) is the dominant isoform expressed in rat taste cells (Clapp et al., 2001). In an attempt to link a possible physiological role to rat taste cell types we used DAB immunoelectron microscopy to determine which cell types express IP₃R3. Our results indicate that a subset of both Type II and Type III cells display IP₃R3 immunoreactivity in their cytoplasm. Type II cells were identified by the presence of multiple microvilli at their apices and their characteristic round nuclei. Several immunoreactive Type II cells were found with subsurface cisternae (SSC) of smooth endoplasmic reticulum at close appositions with nerve fibers. Type III cells were distinguished by the presence of one large apical microvillus and an irregular, invaginated nucleus. We also found evidence for synapses from immunoreactive cells onto nerve processes distinguished by the presence of synaptic vesicles and an electron dense presynaptic membrane. Our data support the idea that a subset of both rat Type II and Type III cells utilize the IP₃ signaling pathway. Supported by NIH grants DC00766, DC00244 and DC00285

ADENYLYL CYCLASES AND CAMP MODULATION IN TASTE BUDS

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G-protein coupled receptors (GPCRs) are involved in sensory transduction of sweet, bitter and umami. Umami transduction is thought to involve a GPCR, taste-mGluR4. In transfected cells expressing this receptor, cAMP levels decrease in response to glutamate (Glu). However, the proximal signaling steps following activation of this receptor in taste buds have not been explored. We measured cAMP levels in intact taste buds in response to Glu. Glu (0.1-20 mM) decreased cAMP in a concentration-dependent manner. The response was augmented by 0.5 mM inosine monophosphate. The enzyme that generates cAMP, adenylyl cyclase (AC) exists as nine membrane-bound isoforms that can be regulated by distinct G proteins. To identify the AC types present in taste cells, we have used RT-PCR with degenerate primers. The products were digested with restriction enzymes with sites that discriminate between different ACs. AC4 and AC5 and possibly also AC 3,6 and/or 9 were present in taste tissue. Immunocytochemistry showed robust signal for AC4 in many cells of all taste buds and strong AC5/6 immunoreactivity in a few taste cells. The AC activator forskolin (10 µM) increased cAMP levels in circumvallate and foliate epithelium by 10-fold. Non-taste epithelium responded less to forskolin stimulation, suggesting that it has either lower titers or distinct isoforms of AC. These studies will allow more detailed characterization of the enzymes involved in signaling umami as well as sweet and bitter stimuli, all of which may modulate cAMP. Supported by NIH (DC03013).

DETECTION OF TASTE QUALITY IN TASTE BUDS

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The process of identifying and distinguishing taste qualities (e.g. bitter versus sweet) begins in taste buds, but the chemical specificity of individual taste cells is still debated. We examined calcium transients elicited by presenting chemical stimuli representing five different taste qualities (bitter: quinine and cycloheximide; sweet: sucrose and saccharin; salt: NaCl; acid: citric acid; umami: monosodium glutamate) to mouse taste receptor cells in lingual slices. Each chemical stimulus we tested induced distinct Ca²⁺ transients, with different time courses and amplitudes. Responses were concentration-dependent. The response thresholds were similar to behavioral thresholds in mice. When tested consecutively with seven different stimuli, 55% of the responsive cells (88 out of 160) responded to only one compound. Sixteen percent of the cells responded to two stimuli, 17% to three stimuli, 7% to four stimuli, 3% to five stimuli, and 2% to six stimuli. Interestingly, we found that 14% of the taste cells responded to both bitter and sweet stimuli. Most responses were independently distributed across taste qualities (chi-square test, $p < 0.001$). Thus, there appears to be no strict and separate encoding of taste qualities into distinct subpopulations of taste cells in the peripheral gustatory organs. Whether activation of specialized subsets of highly selective taste cells is crucial for distinguishing taste qualities depends on how signals from taste buds are transmitted to higher levels of the gustatory system. Supported by NIH/NIDCD grants DC00374 (S.D. Roper) and DC04525 (A.C.).

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF VOLTAGE-GATED CURRENTS IN MOUSE TASTE CELL TYPES

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At least two different cell types reside within a taste bud: dark cells (Type I) and light cells, which can be subdivided into Type II and Type III cells. Extensive research has characterized the presence of certain proteins associated with each cell type, but very little is known about their physiology. In this study we used isolated taste cells to characterize voltage-gated currents found in defined cell types. Cell types were identified by using antibodies to external epitope markers as follows: Type I, Antigen H; Type II, Antigen A; and Type III, NCAM (Pumplin et al., 1999; Takeda et al., 1992). In addition, we identified those Type II cells that contained gustducin by using transgenic mice expressing GFP from the gustducin promoter. Gustducin expressing taste cells had small Na⁺ and K⁺ currents, but no detectable Ca²⁺ current. About half of the Antigen A positive cells had currents similar to those of the gustducin expressing cells. However, the remaining Antigen A positive cells had large Na⁺ and K⁺ currents, as well as a Ca²⁺ current. Ca²⁺ currents were also present in the NCAM positive cells. Antigen H expressing taste cells had small Na⁺ and K⁺ currents and no Ca²⁺ current. Preliminary pharmacological experiments indicate that the Ca²⁺ current in taste cells is carried by several different types of Ca²⁺ channels. Supported by NIH grants DC00766 and DC00244 to SCK and DC03155 to RFM.

SYNAPTOSOMES IS ASSOCIATED WITH SYNAPTIC VESICLES AT TASTE CELL SYNAPSES

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Synaptic vesicle docking and exocytosis of neurotransmitter in the CNS require the interactions of synaptic vesicle proteins (VAMP/synaptobrevin and synaptotagmin) with presynaptic membrane proteins (SNAP-25 and syntaxin). We hypothesize that taste cell synapses utilize the same protein machinery as used by synapses in the CNS. Our preliminary data suggest that immunoreactivity to synaptotagmin, SNAP-25 and syntaxin are all present in taste cells with synapses. In the present study, we wish to determine whether synaptobrevin IR is associated with synaptic vesicles at rat taste bud synapses. Our confocal microscopy results indicate that synaptobrevin IR is present in a large subset of taste cells and nerve processes. Synaptobrevin IR colocalizes with synaptotagmin, SNAP-25 and syntaxin IR respectively in subsets of taste cells. Gustducin IR is also present in a subset of synaptobrevin IR taste cells. Immunoelectron microscopy using DAB reveals that synaptobrevin is present in type II and III taste cells. To date, only type III synaptobrevin IR cells have been observed to synapse with nerve processes. Using colloidal gold immunoelectron microscopy we have observed synaptobrevin IR associated with vesicles at both finger-like and macular synapses from type III cells onto nerve processes. These data suggest that taste cell synapses use the same protein machinery for synaptic vesicle docking and exocytosis as the CNS. Supported in part by NIH grants DC00285 and DC00244.

GENOME-WIDE PROFILING OF INDIVIDUAL TASTE RECEPTOR CELLS

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To identify molecules potentially involved in the morphological and functional heterogeneity of taste cells we set out to establish gene expression profiles for individual taste cells and correlate this molecular data with the cell's phenotype. We used single cell RT-PCR, differential screening and bioinformatics to identify a number of genes that may be involved in taste cell signaling, coding and turnover. In certain subsets of taste cells we noted patterns of coordinate gene expression. Amongst clones from single taste cells we identified signal transduction components, development- and differentiation-related genes, transcription factors, sequences with est (expressed sequence tag) matches, and novel sequences. Real time-PCR indicated that some of these genes are selectively expressed in taste tissue. Genes that are preferentially represented in RNA from taste tissue vs. non-taste tissue were picked and rearranged to make microarray "taste chips." Single taste cell cDNA probes were prepared by a single cell RT-PCR method which largely preserved the original representation of mRNA abundance in the initial cell. Taste chips were screened with amplified single cell cDNAs to generate single taste cell gene expression profiles, which were then validated by in situ hybridization and immunostaining. We expect that this approach will provide us with a molecular genotype to correlate with a taste cell's phenotype. Supported by NIH grants DC03155 (RFM), MHS7241 (MM) and DC00310 (LH). RFM is an Investigator of the Howard Hughes Medical Institute.

NEUROTROPHIN RECEPTORS IN RODENT TASTE BUDS

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The trk family of tyrosine-protein kinase receptors: trkA, trkB and trkC are high affinity receptors that bind the neurotrophins: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and NT4/5. Neurotrophins and their receptors classically are thought to be important for the proper development, innervation and survival of the nervous system. Previous studies have established that BDNF is present not only in developing, but also in adult taste buds. To determine whether adult rodent taste cells express neurotrophin receptors, antibodies directed against trkA, trkB or trkC were applied to adult mouse and rat gustatory tissue. In addition, RT-PCR analysis was used to test for the presence of trkA, trkB and trkC transcripts. Immunocytochemistry shows that trkA-immunoreactive (IR) elongate taste cells are present; whereas trkB-IR and trkC-IR taste cells were not detected. For RT-PCR studies, taste buds were isolated from rat fungiform, foliate and vallate papillae, and total mRNA was extracted from the isolated taste buds. RT-PCR analysis indicates that trkA, but not trkB or trkC mRNA is present in adult taste buds. In summary, immunohistochemical and RT-PCR data are congruous, indicating the presence of both the mRNA and protein for trkA but neither mRNA nor protein for trkB or trkC in adult taste cells. Supported by NIH grant DC00244 to T.E.F. and DC04837 to A.I.F.

STRUCTURE AND FUNCTION OF FUNGIFORM TASTE BUDS IN BAX-KNOCKOUT MICEYuskaitis C.J.¹, May O.L.¹, Hill D.L.¹ ¹*Psychology, University of Virginia, Charlottesville, VA*

Apoptosis, a vital mechanism in the development and maintenance of tissues in the vertebrate gustatory system, is promoted by a cascade of signals that include Bax, a death factor in the *bcl-2* family of survival/death factors. In mouse, *bax* plays a role in controlling the size of vallate taste buds by mediating taste bud cell number. Oakley and colleagues (2000) demonstrated that *bax* knockouts had a marked increase in the number of vallate taste bud cells compared to wild-type controls. It is hypothesized that *bax* also affects fungiform taste bud structure and function. In order to identify changes in fungiform taste bud volume size, cytokeratin 8 (TROMA) immunohistochemical procedures were used on sectioned adult mouse tongues. Taste bud volumes were determined (Neurolucida software) and compared between genotypically identified *bax* ^{-/-} and wild-type mice. Preliminary results indicate an approximate 1.5X increase in taste bud volume in the *bax* knockout mouse. However, this increase in volume was not accompanied by an increase in taste bud number. Functionally, chorda tympani nerve responses to a concentration series (0.05M – 0.5M) of NaCl, ammonium chloride, KCl and sodium acetate and to single concentrations of quinine, HCl and sucrose were similar between groups ($p > 0.10$). These data indicate that the *bax* gene plays a considerable role in fungiform taste bud morphology without changes in taste bud numbers and in neurophysiological responses. Supported by NIH grant DC00407 and U.Va. Harrison Foundation.

TOWARD A NOMENCLATURE FOR HUMAN AND MOUSE OLFACTORY RECEPTORSCrasto C.J.¹, Shepherd G.M.¹ ¹*Neurobiology, Yale University, New Haven, CT*

With the publication of the first draft of the Human Genome, three groups have independently identified the olfactory receptor genes, which total more than 950. Of these, approximately 350 have been identified as functional. Recently the Mouse Genome has become available, in which the olfactory receptor genes total 1296; of these 1032 are functional. This tremendous amount of new data presents a problem for developing a satisfactory nomenclature. Developing a nomenclature is however critical for unambiguous identification of specific receptors for further experimental and theoretical studies. The Olfactory Receptor Database (ORDB) at senselab.med.yale.edu has been designated as a nodal point for assisting the field in the development of such a nomenclature. To bring together the data on the human and mouse olfactory receptors, we are tentatively exploring the possibility of a nomenclature in which categorization is based on chromosomal location, with numbering of individual sequences. For the human the numbering reflects the consensus of the reporting laboratories. For the mouse it represents the order reported. The result of the sequence analysis and the nomenclature can be found at <http://senselab.med.yale.edu/senselab/ORDB/orseqanal.html>. Discussion with the NIDCD Advisory Committee and workers in the field will lead to a final consensus. Supported by NIH grants DC00086, DC52550 (Human Brain Project), and MURI (DOD).

DEVELOPMENT OF AN IN VIVO EXPRESSION SYSTEM FOR FUNCTIONAL ANALYSIS OF INSECT OLFACTORY PROTEINSRogers M.E.¹, Peterlin Z.A.¹, Chesler A.T.¹, Firestein S.¹ ¹*Biological Sciences, Columbia University, New York, NY*

The highly organized structure of insect antennae has facilitated the identification of many olfactory-relevant genes. Functional characterization of many of these genes has remained elusive in the absence of an adequate expression system. We are developing such a system in the moth *Manduca sexta* whereby olfactory proteins are transiently expressed in antennal cells using recombinant baculoviruses. Antennal injection of a baculovirus containing GFP cDNA yielded robust GFP protein expression in support cells. Single unit electrophysiological recordings of infected sensilla indicated that the associated olfactory receptor neurons (ORNs) were viable and capable of responding to odorant stimuli. However, few GFP+ ORNs were visualized in these intact preparations, presumably due to limited access of the virus to these cells. Baculovirus-accessible ORNs were obtained by culturing dissociated antennae under conditions favoring ORN maturation and survival. Infection of 10-14 day cultures with baculoviruses containing GFP, *Antheraea* SNMP, or a *Drosophila* odorant receptor (OR) cDNA followed by immunocytochemical detection indicated robust expression of these proteins in ORNs. Calcium imaging studies confirm that infected ORNs are capable of responding to external stimuli; KCl and odorant mixtures yielded increases in intracellular Ca²⁺ levels ($\% \Delta F/F > 2$). These preliminary studies suggest that the *M. sexta* antenna may provide an adequate expression system to examine the functional properties of support cell (e.g. OBP) and neuronal (e.g. OR, SNMP) proteins derived from a diversity of insect species.

COMPARISON OF HOMOLOGOUS AND HETEROLOGOUS EXPRESSION SYSTEMS FOR OLFACTORY RECEPTORSBieri S.¹, Monastyrskaja K.¹, Valero A.¹, Schilling B.¹ ¹*Bioscience, Givaudan Dubendorf Ltd, Dubendorf, Switzerland*

Heterologous cell systems are widely used to express and characterize functional G protein-coupled receptors (GPCRs) on the cell surface in vitro. The olfactory receptors (ORs), the largest family of GPCRs, are unusual since expressed recombinant receptors are not readily transported to the cell surface of heterologous cells. Consequently, the percentage of functional ORs that are accessible to odorants is low. Various conditions were optimized to improve the heterologous expression of ORs in a mammalian cell line (HEK293) by means of transfection or infection. Increasing the number of functional receptors on the cell surface is a prerequisite for studying their ligand specificity. Rat olfactory receptor neurons (ORNs) were used as host cells for homologous expression of ORs, since receptor transport to the cell surface is ensured in those cells. The rat OR I7 was expressed in dissociated mature ORNs and activation by ligands was monitored by calcium imaging. The ligand specificity of the recombinant I7 was consistent with data in the literature. Activation of dissociated mature ORNs by odor molecules of the sandalwood family was studied by calcium imaging. It was possible to identify ORNs showing specific responses to structurally related sandalwood compounds but not unrelated odorants. The disadvantage of using mature ORNs is that they do not survive for a long time in culture. Therefore we have been looking at the possibility of using immature ORNs which can be kept in culture for up to ten days. Preliminary data suggested that these cultured neurons can be stimulated by odorants similar to mature ORNs.

253 Poster Olfactory Receptors: Genes and Proteins**FUNCTIONAL CHARACTERIZATION OF MOUSE ODORANT RECEPTOR, MOR-EG: EFFECTS OF RECEPTOR MODIFICATION AND MUTATIONS**

Katada S.¹, Tanaka M.¹, Nakagawa T.¹, Kajiya K.¹, Kataoka H.¹, Touhara K.¹ ¹*Department of Integrated Biosciences, University of Tokyo, Chiba, Japan*

We recently demonstrated the functional reconstitution of mouse odorant receptors in HEK293 cells, providing molecular evidence that structurally-related olfactory receptors recognize overlapping sets of odorants with distinct ligand specificities [1]. Using the mouse odorant receptor, mOR-EG, and its cognate ligands, eugenol, vanillin, and ethyl vanillin, we performed Ca²⁺ response assays in various mammalian cell lines (HEK293, COS-7, CHO-K1, and PC12) and in *Xenopus* oocytes. Addition of a rhodopsin-tag on the N-terminus of mOR-EG appears to enhance efficiency of mOR-EG functional expression, but this modification was not crucial for its odorant-response activity. FLAG-tag or GFP additions at the C-terminus of the receptor, however, significantly reduced the odorant-response activity. Immunocytochemical analyses of the modified receptors revealed that the odorant-response activity had no correlation with subcellular localization of the receptors. Finally, mutations of single amino acid residues within TM3 and TM5 of mOR-EG dramatically affected the EC50 values of the ligands, indicating that the odorant-binding site resided within the transmembrane portions of the receptor. The current studies provide caveats for successful odorant receptor expression in heterologous systems, and suggest a mode of binding to its cognate ligands. [1] Kajiya et al. (2001) *J.Neurosci.* 21.6018-6025

254 Poster Olfactory Receptors: Genes and Proteins**CHARACTERIZATION OF A FAMILY OF CANDIDATE ODORANT RECEPTORS FROM THE MALARIA VECTOR MOSQUITO ANOPHELES GAMBIAE**

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Olfaction plays an important role in many behaviors, including feeding, mating and socialization, of many organisms. Our laboratory is interested in the molecular biology of olfaction as it impacts upon host selection in the major human malaria vector mosquito, *Anopheles gambiae*, where dissecting the olfactory signaling cascade may lead to novel disease-prevention strategies. One focus involves cloning and characterization of odorant receptors that are presumed to initiate olfactory signaling in *A. gambiae*. Molecular and bioinformatics-based approaches have been used to identify a large family of candidate odorant receptors in *A. gambiae* (AgORs). Of these, a considerable number display significant homology to members of the *Drosophila melanogaster* odorant receptor family, while others appear to be unique to *A. gambiae*. Spatial and developmental expression data will be discussed, along with expression patterns of putative AgORs in response to blood feeding. Future projects include immunolocalization of candidate AgORs within the olfactory system of *A. gambiae*, as well as heterologous expression of a subset of AgORs in order to identify activating odorants. Eventually, in order to address questions regarding the role of AgORs in host preference, this study will include an interspecific comparison of odorant receptors from anthropophilic vector mosquitoes and zoophilic non-vector species of Anopheline mosquitoes.

255 Poster Olfactory Receptors: Genes and Proteins**MOLECULAR STUDIES OF HUMAN OLFACTORY RECEPTOR NEURONS**

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Studies of olfactory transduction during the last decade have concentrated on the function of isolated ORNs. Due to the limited availability of freshly isolated human ORNs, we have grown human olfactory cells in culture and begun to characterize them using morphological, functional and molecular approaches (Gomez et al., 2000). The most well-characterized pathway for olfactory signal transduction involves activation of G proteins (Golf), followed by cAMP production and activation of cation channels (cyclic nucleotide-gated channels, CNC). In these studies we used immunohistochemistry and in situ hybridization to examine the expression of two components of the cAMP signal transduction pathway in these long-term, primary cultures. Our data show: a) only morphologically defined olfactory receptor neurons express mRNA for olfactory marker protein (OMP), Golf and CNC compared with supporting cells. b) Approximately 80% of the cells characterized as olfactory neurons on the basis of their characteristic morphology co-express OMP and Golf; OMP and CNC or Golf and CNC. c) Immunoreactivity for Golf is consistent with the result of in situ hybridization using an RNA probe for Golf. RT-PCR and in situ hybridization studies also demonstrated expression of specific olfactory receptor genes. The availability of this model system will enable a complete molecular characterization of the human olfactory receptor neuron and provides a tool to further investigate the regulation of gene expression during proliferation and maturation. Funded in part by NIH 00214 and NIH 00566.

256 Poster Olfactory Receptor Neurons: Functional Properties**SINGLE-UNIT RECORDING FROM GOLDFISH OLFACTORY RECEPTOR NEURONS USING A WIDE RANGE OF BIOLOGICALLY RELEVANT ODORANTS SUGGESTS HIGH CHEMOSPECIFICITY**

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Although many studies suggest vertebrate olfactory receptor neurons (ORNs) are tuned to one/few odorants, there have been few direct tests of this possibility owing to a paucity of identified primary odorants and their receptors. Here, we describe preliminary findings of such a test using the goldfish for which 6 distinct classes of biologically relevant odorants have been identified (amino acids, amines, and nucleotides [feeding stimuli]; bile acids [social aggregants]; F prostaglandins and sex steroids [sex pheromones]). We are employing single-unit in vivo extracellular recording and low concentrations of mixtures of representative odorants from each odor class. Results to date (n=15), find most ORNs (12/15) to respond to at least one odor mixture (suggesting we know most relevant odors for this species), with the vast majority of ORNs responding to just one class (10/12). Excitation appears to be the most common response unlike the case for amino acid sensitivity in catfish. Most pheromone-sensitive ORNs appear to be located near the central raphe regions of the lamellae where microvillar and crypt ORN distribution is greatest, corroborating multi-unit recording data (Sorensen et al. this symposium). Tests of tuning to individual components are planned by the time of the meeting. NIH/DC03792.

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UPDATE ON POLYAMINES AS OLFACTORY STIMULI IN GOLDFISH

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We reported last year that suprathreshold concentrations of putrescine, a polyamine, were significantly more potent olfactory stimuli to goldfish than the most stimulatory amino acid, L-arginine (Arg). We report here that the magnitudes of EOG responses to 0.1mM putrescine, cadaverine and spermine are 4.2x, 4.7x and 14.1x, respectively, that of the standard, 0.1mM Arg, the most potent amino acid. Cross-adaptation experiments indicate the independence of olfactory receptor sites for polyamines from those to amino acids (L-isomers of Arg, alanine, methionine and glutamate), bile salts (Na⁺ taurocholate and tauroolithocholate), single amine compounds (amylamine and butylamine) and ATP. Further, the cross-adaptation experiments indicated that receptor sites for the individual polyamines are relatively independent from each other. EOG recordings were obtained to mixtures of polyamines, amino acids and bile salts, prior to, during and after the adenylyl cyclase activator, forskolin (1-20 μ M), bathed the olfactory organ. During adaptation to forskolin, EOG responses to bile salts were eliminated, while responses to amino acids were only partially attenuated. Responses to polyamines, however, were least affected. These results suggest that polyamine odorants are transduced by a non-cAMP second messenger pathway; however, bile salt transduction likely involves the cAMP pathway, confirming previous data from zebrafish (Michel, 1999). We are currently attempting to determine whether the IP3 second messenger pathway is involved in olfactory polyamine transduction. Supported by NIH DC-03792

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METABOLIC PROFILE AND ODOR RESPONSIVENESS OF SQUID OLFACTORY NEURON SUBTYPES

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The squid olfactory organ consists of five olfactory receptor neuron (ORN) types characterized by their morphology. We determined the metabolic profiles of the cell types using amino acid specific antibodies, so we could assay the odor responsiveness of identified cell types using the activity marker agmatine (AGB)¹. In the presence of an odor, externally applied AGB enters activated cells through non-selective cation channels. We exposed squid, *Lolliguncula brevis* to various odors plus AGB *in vivo*. The expression profiles of six amino acids were examined in fixed, embedded and sectioned (50 nm) olfactory organs. Serial sections stained for each amino acid were captured as 8-bit gray-scale images using 20X or 100X objectives. Following digital registration, cluster analysis was used to identify the cell types and quantify the amino acid contents. Images were analyzed for cell types and pixel area that had AGB labeling. Of 502 cells identified based on their metabolic profiles (10 preparations), 0.8% were type 1, 32% type 2, 26% type 3, 15% type 4 and 19% type 5. Odor-stimulated AGB labeling ranged from 0.8% in type 1 cells to 11% in type 3 cells. On average, odors stimulated AGB labeling of about 3% of the olfactory epithelium. Glutamate (50 μ M) stimulation resulted in the largest percent area of AGB labeling. These data suggest that the 5 cell types differ in their relative abundance, odor responsiveness and metabolic profiles, as well as in their morphology. This research was funded by NIH NINDS grant# P01 NS07938 to MTL and WCM. 1. Michel et al., *J Neurosci Methods*. 1999 90(2):143-56.

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STATISTICAL METHOD FOR RAPID ANALYSIS OF OLFACTORY NEURON ACTIVITY

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The analysis of single spontaneously active neuron response to stimulation requires the detection of neuron activity change. A statistical method (CSA), based on analysis of the cumulative distribution slope, was developed for detection of activity change. The slope of the cumulative distribution of spike times estimates neuron activity expressed as a spike firing rate. During the pre-stimulation period, spontaneous firing rate can be estimated as the cumulative distribution slope at each spike time. The distribution of expected local firing rates during spontaneous activity is thus provided, showing the range in which the firing rates of non-responding neuron are expected. Since the slope of cumulative distribution depends upon the local density of spikes, a change of activity can be detected as exceptionally high slope (in the case of excitation) or exceptionally low slope (in the case of suppression). Besides the type of response, the response onset, magnitude and duration are reported. The response magnitude is expressed as how many times larger (smaller) than expected is the detected firing rate during excitation (suppression). The CSA graphically presents the raster plot of original data, their cumulative distribution and local firing rate estimates. The method was used for batch analysis of large number (more than 400) single unit recordings. The typical analysis time on moderate computer, producing the graphs and table of results, was less than 2s per single unit recording. A modified version could be used for on-line analysis and activity change tracking. Partly supported by MSZS J1-3366-0105-01.

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MULTIDRUG RESISTANCE TRANSPORTERS IN OLFACTORY RECEPTOR NEURONS OF XENOPUS LAEVIS TADPOLES

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Olfactory receptor neurons (ORNs) are the only class of neurons that are directly exposed to the environment. Therefore they need to deal with xenobiotic and potentially cytotoxic substances. Here we show for the first time that ORNs possess transporter systems that expel xenobiotics across the plasma membrane. Using calcein and Ca²⁺-indicator dyes as xenobiotics we demonstrate that ORNs appear to express the multidrug resistance P-glycoprotein (MDR1) and multidrug resistance-associated proteins (MRP). This endows ORNs with the capability of transporting a large number of substrates across their plasma membranes.

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SUPPRESSING OLFACTORY SENSORY NEURON (OSN) ACTIVITY WITH ULTRAVIOLET (UV)-LIGHT

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We have developed a method for targeted suppression of OSN activity, measured using the electroolfactogram (EOG), by exposure of amphibian olfactory epithelium (OE) to UV-light (peak emission at 254 nm). Properties of this method include recovery requiring 12 hrs, the level of EOG suppression increasing with UV dose, and EOG responses to low concentrations eliminating before those at higher concentrations. Blockade occurred regardless of location on the OE and was observed for all odorants tested: amyl acetate, geraniol, ethyl fenchol, and (-)-carvone, concentrations from 10^{-1} – 10^{-3} sat. vapor. The OE can be protected from UV-induced suppression by shielding. Total UV exposure determined the level of attenuation: a 10-min. continuous exposure was equivalent to 5 exposures for 2-min. each. Since blockade is independent of odorant type, concentration, and OE site, there may be a single mechanism underlying the UV-induced effect. As has been shown *in vitro* (Middendorf and Aldrich, 2000, J. Gen. Physiol. 116(2):253-82), UV affects the current carrying capacity of the cyclic-nucleotide gated channel, suggesting this may be the molecular mechanism for EOG blockade. These findings indicate that EOG blockade by UV is a robust method for investigating how targeted suppression of OE responses affects bulbar activity based on patterns of olfactory receptor expression (Marchand et al., AChemS XXIII) and regional differences in OE responses. Supported by NIDCD.

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DIFFERENTIAL RESPONSIVENESS OF CILIATED AND MICROVILLAR OLFACTORY RECEPTOR NEURONS IN GOLDFISH

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Although odor information in fish is detected by at least 3 different types of olfactory receptor neurons (ORNs) (ciliated, microvillar and crypt), little is known about the specificities of these cell types. We explored this question in goldfish by means of correlated histological and electrophysiological approaches. Immunohistochemical studies found ciliated ORNs expressing G_{olf} distributed across the entire surface of the sensory epithelium including both the regions adjacent to the raphe (peri-raphe) and the outer regions (marginal) of the lamellae. In contrast, microvillar and crypt ORNs expressing either G_o or G_q were concentrated in peri-raphe regions. Multi-unit *in vivo* recordings found all regions of all lamellae to be consistently sensitive to amino acid stimuli (74 of 74 loci). In contrast, ORNs responding to identified sex pheromones (17,20 -dihydroxyprogesterone and/or 15-ketoprostaglandinF₂) were plentiful in peri-raphe regions, but were relatively uncommon (<20% of loci) in the marginal regions where ciliated ORNs predominate. In conclusion, the distribution of ORNs that respond to sex pheromones suggests that microvillar and/or crypt ORNs mediate responses to these cues while some ciliated ORNs likely respond to amino acids alone. Supported by NIH/DC03792; NSF/IBN9723798

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FUNCTIONAL AND BIOCHEMICAL DIFFERENCES BETWEEN MICROVILLAR AND CILIATED OLFACTORY RECEPTOR NEURONS IN CATFISH

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The olfactory system of vertebrates encompasses ciliated (cORN) and microvillar receptor neurons (mvORN) projecting to different territories in the extended olfactory bulb (OB). The cORNs and mvORNs utilize different families of receptor molecules (OR, V1R, V2R) coupled to different G-proteins. In fishes, unlike rodents, the different morphological types of receptor cells are intermingled in a single sensory epithelium but nonetheless project to different territories in the OB. We used anatomical, molecular and electrophysiological methods to test whether in catfish a correlation exists between the morphology of the ORN, the molecular transduction system and the type of odorant detected. Electrophysiological tests with 3 types of biologically relevant stimuli revealed that ORNs detecting bile salts project to the medial middle part of the OB. Retrograde DiI tracing from this area identified these as cORNs which utilize G_{olf}. Likewise, recordings from the anterior dorsal and the ventral side of the OB showed that these areas mainly process amino acid odor information and receive input from both mvORNs and cORNs. Nucleotide responses are present in the posterior dorsal part of the OB which receives input from mvORNs. Our experiments suggest that two different types of ORNs (ciliated and microvillar) detect different classes of odorants: bile salts by cORNs and nucleotides by mvORNs. Supported by NIH Grant DC03792 (J.C., P.I.)

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CON A SELECTIVELY BLOCKS DETECTION OF CARVONE ENANTIOMERS IN WISTAR RATS

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Previous investigations revealed that intranasal application of the lectin concanavalin A (Con A) reduces odor detection ability in rats for some but not all odors tested. The present experiment investigated the effect of Con A on D- and L-carvone detection ability. Male Wistar rats were trained to respond to the odor of carvone using operant conditioning procedures. Animals were divided into three groups. Group 1 was trained to detect D-carvone, group 2 was instructed to detect L-carvone and group 3 was able to generalise across L- and D-carvone. During behavioral studies rats were kept on a 23,5 h water-deprivation schedule. The results revealed that in group 1 at the concentration 2 mg/ml Con A reduced D-carvone detection ability in all animals ($57 \pm 5\%$ cr, $p < 0.01$). To analyse whether the sugar specific binding site of Con A is responsible for the inhibitory effect on D-carvone detection animals were treated with Con A preincubated with 0.1 M α -methyl mannoside. D-carvone detection after sugar incubation of Con A was significantly higher compared to Con A without α -methyl mannoside ($p < 0.05$). Thus, the sugar binding site of Con A seems to be responsible for the reduced D-carvone detection after lectin treatment. In group 2 no effect on L-carvone detection was evident either at 2 ($90 \pm 5\%$ cr) or 4 ($87 \pm 8\%$ cr) mg/ml Con A. In group 3 neither D-carvone nor L-carvone detection was affected by Con A ($98 \pm 2\%$ cr) in contrast to animals in group 1 which only knew D-carvone as the rewarded odor. The findings are discussed.

POLYMORPHISMS IN AN ACID SENSING ION CHANNEL (hfpASIC1) IN HUMAN FUNGIFORM PAPILLAE

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Acid sensing ion channels (ASICs) are a family of proton-gated ion channels, one member of which (hfpASIC1) has been cloned by us from human fungiform papillae and shown to act as a sourness detector. In this study we explored the occurrence of polymorphisms in hfpASIC1. Fungiform papillae were biopsied from volunteers. hfpASIC1 was amplified by RTPCR, subcloned and sequenced. Complementary RNA was prepared and injected into frog oocytes. The pH was rapidly lowered from neutrality to 5.5 and the response measured by 2-electrode voltage clamp analysis. Two subjects (# 2 & 4) expressed hfpASIC1 with no sequence variations. When the cRNA of these subjects was injected into oocytes, normal characteristic and amiloride sensitive responses were seen to pH 5.5. Two subjects (# 18 & 62) showed no detectable expression of hfpASIC1. One subject (#1) expressed hfpASIC1 with a 32 bp deletion in the region of the predicted first transmembrane domain. The cRNA of this individual showed no response to pH 5.5. Using only the tips of their tongues, Subjects #18, 62 & 1 were unable to identify as sour a solution of 15 mM citric acid. Another subject (#25) expressed hfpASIC1 with a 82 bp insertion in the region of the predicted extracellular domain. The cRNA of this subject did not respond to pH 5.5 when tested in oocytes. However, this subject was able to identify the sourness of citric acid in tip-of-the-tongue tests, as did Subjects #2 & 4. These data provide the first evidence for the presence of polymorphisms in hfpASIC1. Supported in part by a grant from the Dept. of Veterans Affairs (to JGB).

TRANSCRIPTION OF ENAC SUBUNITS IN THE DEVELOPING RAT

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An underlying molecular alteration of fungiform taste cells correlating to the functional augmentation of sodium sensitivity during development has not been elucidated. The developmental increase in sodium response of the chorda tympani nerve is related to a change in amiloride-sensitive epithelial sodium channel (ENaC) concentration and efficiency in taste receptor cells. A semi-quantitative RT-PCR approach has been taken in order to determine if regulation of ENaC in fungiform taste cells is a transcriptional event. Densitometric measurements were taken of RT-PCR products from pooled fungiform taste cell RNA during postnatal development and standardized to GAPDH expression. Preliminary evidence indicates that the development of sodium sensitivity is not dependent on the absence and subsequent initiation of specific subunit transcription, however, it may involve a quantitative change in transcription of one or more of the three ENaC subunits. These data will allow for the correlation of molecular events with a functional consequence in the gustatory system. Funded by NIH grant DC00407

CHARACTERIZATION OF ACID SENSITIVE ION CHANNELS (ASICs) IN MOUSE TASTE CELLS

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ASICs are members of the amiloride-sensitive Na⁺-channel/degenerin family that form proton-gated ion channels. They are expressed in sensory neurons and the central nervous system where they are important in acid-induced nociception. Because of their acid sensitivity, ASICs are candidates for playing a role in sour taste transduction. Previous work in our lab using electrophysiological techniques identified an acid sensitive current in rat taste cells with properties similar to known ASIC currents. To determine which ASIC channels may be expressed in taste cells, we used RT-PCR on total RNA isolated from mouse circumvallate papillae (CV) with primers specific for ASIC1, ASIC2a, ASIC2b, ASIC3 and ASIC4. Preliminary results indicated PCR products of the appropriate size for ASIC2a, 2b, and 4. No products were obtained for ASIC1 or ASIC3, but RNA from positive control tissues produced products of the appropriate size. PCR products were then subcloned and sequenced, which confirmed initial findings. Immunocytochemistry was performed on fixed mouse CV sections using antibodies specific to ASIC1 and ASIC2a. Heavy taste cell pore labeling was seen with ASIC2a, while no labeling was seen with ASIC1. Western blotting of protein from mouse CV has also been consistent with these results showing the expression of ASIC2a but not the presence of ASIC1. Further studies will be required to examine expression patterns of ASIC2b and 4. Supported by NIH grants DC00766 and DC0024 to SCK.

EVIDENCE FOR EXPRESSION OF TASK-LIKE K⁺ CHANNELS IN RAT TASTE CELLS

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In whole-cell recordings of rat vallate taste cells, we found a subset of cells with three striking features: a highly negative resting potential of -71.1 ± 1.2 mV (n=20), a relatively large membrane conductance, and high sensitivity to acids. A drop in extracellular pH from 7.4 to 7.2 and below decreased the membrane conductance, resulting in a sustained depolarizing current. Further, the acid-sensitive conductance reversed near the K⁺ equilibrium potential and was blocked by BaCl₂, but not by TEA. These features resemble the recently cloned TASKs (Twik-related acid-sensitive K⁺ channels; Lesage and Lazdunski, *Am J Physiol Renal Physiol* 2000, 279:F793-801). To verify expression of TASK-like channels, we labeled rat taste buds with antibodies against TASK-1 and TASK-2. Positive reaction was detected for TASK-2, with strong label in some taste cells and weak label in many cells. We also designed primers for mammalian TASK-1 and TASK-2 and probed mRNA isolated from taste buds of fungiform, foliate and vallate papillae using RT-PCR. Consistent with the immunocytochemical staining, TASK-2 was highly expressed in all three types of taste buds. The TASK-1 message, while detectable, was apparently expressed at a much lower level than TASK-2. These data suggest that TASK-like K⁺ channels are present in rat taste cells and may be involved in setting resting potentials and in sour taste transduction. Supported by NIH grants DC00766, DC00244 (SCK), DK59611 (TAG).

ACUTE REGULATION OF RAT NaCl TASTE RESPONSES BY pH

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We investigated the pH-dependence of rat chorda tympani (CT) nerve responses to NaCl and of intracellular Na⁺ activity ([Na⁺]_i) in polarized TRCs. Tongues were stimulated with NaCl solutions buffered to pH 2, 7, and 10. The NaCl CT responses were inhibited at pH 2 and were enhanced at pH 10 relative to responses at pH 7. The voltage sensitivity of the NaCl response was decreased by 50% at pH 2 but enhanced by 45% at pH 10 relative to pH 7. Benzamil inhibited NaCl responses at pH 7 by 75%. The remaining benzamil-insensitive response was unchanged at pH 2 or 10. However, at constant external pH the response with NaCl in acetic acid (AA)/potassium acetate (KA) buffer (pH 6) or with NaCl in HCO₃⁻/CO₂ buffer (pH 7) was 50% smaller compared to control using HEPES buffer at pH 6 or 7, respectively. This inhibition with membrane-permeable weak acids (AA or CO₂) suggests a primary role for intracellular pH (pH_i). TRC pH_i decreased with (i) lowering apical pH from 7.4 to 2, and (ii) replacing apical solution with a similar solution buffered to pH 7.4 with KA/AA or with HCO₃⁻/CO₂. At pH 10 pH_i increased. The [Na⁺]_i decreased with (i) lowering apical pH from 7.4 to 3 (ii) apical benzamil, and (iii) apical Na⁺ removal. At pH 7.4 during a basolateral NH₄Cl pulse there was an initial increase in [Na⁺]_i during the alkalization phase, and upon NH₄Cl washout, a decrease in TRC [Na⁺]_i during the acidification phase. We conclude that TRC pH_i regulates Na⁺-influx through amiloride-sensitive ENaC and hence modulates NaCl CT responses in acid/salt mixtures. Supported by NIDCD grants DC-02422 and DC-00122.

ACUTE REGULATION OF RAT NaCl TASTE RESPONSES BY cAMP AND CALCIUM

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Acute regulation of salt taste responses by cAMP and Ca²⁺ was studied by monitoring rat chorda tympani (CT) nerve responses to NaCl and resting intracellular Na⁺ activity ([Na⁺]_i) in polarized Taste receptor cells (TRCs). Topical lingual application of membrane permeable 8-chlorophenylthio (CPT)-cAMP (20 mM in DMSO) for 1 hr enhanced NaCl CT responses relative to control. Amiloride (100 mM) inhibited cAMP-induced increase in NaCl CT responses. Relative to responses at zero current-clamp (0 CC), NaCl CT responses were enhanced at -60 mV and suppressed at +60 mV. A Change in voltage from -60 to +60 mV induced a greater suppression in CT response in presence of cAMP than in its absence. These effects were not observed with 8-CPT-cGMP. We conclude that an acute increase in TRC cAMP increases NaCl CT responses. Topical lingual application of the Ca²⁺ ionophore, ionomycin (150 mM in DMSO) + 10 mM CaCl₂ inhibited the NaCl CT responses^{***} relative to control. Further stimulating the tongue with NaCl + 100 mM amiloride only slightly increased the inhibition relative to NaCl alone. The topical application of DMSO or CaCl₂ alone did not affect CT responses. We conclude that an increase in [Ca²⁺]_i inhibits amiloride-sensitive part of the NaCl CT response. In polarized TRCs cAMP increased and ionomycin decreased unilateral amiloride-sensitive apical Na⁺ fluxes providing the physiological basis of cAMP and Ca²⁺ effects on NaCl CT responses. We conclude that Na⁺ taste sensitivity is subject to acute changes in TRC cAMP and Ca²⁺. Supported by NIDCD grant DC-02422

CPC-SENSITIVE SALT TASTE RESPONSE IN RAT: DOSE-RESPONSE AND VOLTAGE-SENSITIVITY

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Cetylpyridinium chloride (CPC) below 0.25 mM enhances the amiloride-insensitive (AI) rat chorda tympani (CT) response to NaCl. Above 0.25 mM, CPC inhibits the AI NaCl response with maximal inhibition at 2 mM. KCl and NH₄Cl have CPC concentration-response relations similar to NaCl's, but with 60% of the response as CPC-insensitive. We investigated the effect of 0.25 mM CPC on the voltage sensitivity of the CT response to each of the salts in the presence of amiloride. At 100 mM, KCl had a voltage-sensitivity index (VSI) of 0.16±0.03. This increased to 0.43±0.05 in 0.25 mM CPC. Significant increases in VSI with 0.25 mM CPC were observed up to 0.8 M KCl with similar results for AI NaCl and NH₄Cl responses. The increased voltage sensitivity of the responses in the CPC-treated cases suggests that the CPC-modulated membrane transporter is a cation selective apical membrane ion channel. Consistent with this, the CT responses for KCl, NH₄Cl, and the AI part of the NaCl response were saturable functions of their electrochemical concentrations. This was demonstrated directly using a polarized fungiform taste receptor cell (TRC) preparation loaded with Na-green. Adding 25μM CPC to the apical side increased TRC Na⁺ (increased Na⁺ entry) and adding 2 mM CPC decreased TRC Na⁺ below its resting level (inhibition of Na⁺ entry). Similar activation-inhibition results were obtained for TCR K⁺ and NH₄⁺ ions. We conclude that the bimodal effects of CPC are related to either activation (< 0.25 mM) or inhibition (> 0.7 mM) of apical ion fluxes. Supported by NIDCD grant DC-02422.

GLOSSOPHARYNGEAL NERVE TRANSECTION DOES NOT COMPROMISE CHLORIDE SALT DISCRIMINATION IN THE RAT

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We previously reported that rats were clearly able to discriminate between NH₄Cl and KCl despite well-documented similarities in the taste qualities of the two salts. Moreover, the discrimination was not compromised by addition of the epithelial sodium channel blocker amiloride, but dropped to chance levels when the gustatory branches of cranial nerve VII were transected. We tested whether transection of the glossopharyngeal nerve (GL), which carries afferents from over 60% of the taste buds in the rat's oral cavity, might also affect NH₄Cl vs. KCl discrimination. A two-lever operant conditioning procedure was used to train water-restricted rats to discriminate either NH₄Cl from KCl (n=7) or NH₄Cl from NaCl (n=8). Correct responses were rewarded with water. On average, discrimination performance for both groups was 90% or better. GL transection had no effect on the % correct for either group. Amiloride (100 μM) also failed to compromise NH₄Cl vs. KCl performance either before or after GL transection, but significantly impaired both pre- and post-surgical NH₄Cl vs. NaCl discrimination performance (ps < 0.05). These results support our previous findings that NH₄Cl and KCl are discriminable to the rat even in the absence of amiloride-sensitive taste receptor activity. Our results also lend further credence to the hypothesis that in rats input from the 7th cranial nerve is more important for the perception of taste quality than input from the GL, despite innervating less than half the number of taste buds. This work was supported by NIDCD grants: F31-DC05107 and R01-DC01628.

SOLID PHASE MICROEXTRACTION HEADSPACE ANALYSIS OF URINARY VOLATILES FROM ADULT MALE MOOSE

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Mammalian scent marking serves many functions. In male cervids, such as moose (*Alces alces*) scent-marking by urination (scent-urination) occurs during the mating season. Scent-urination in adult male moose is directed more towards females than males because female moose are strongly attracted to the odoriferous urine of rutting males. Based on the odoriferous nature of rut urine, we tested the hypothesis that urine from adult male moose during rut would contain more volatile compounds in elevated levels than urine from the pre-rut, and post-rut periods. Samples consisted of urine from pre-rut (early September), rut (late September-early October), and post-rut (late-March) collected from 3 adult male moose. The headspace of 25 ml of urine from the pre-rut, rut, and post-rut periods was sampled using Solid Phase Microextraction (SPME) and analyzed by GC/MS. Statistical analysis of the major volatile peaks for the 3 periods was performed by using ANOVA ($p < 0.05$) (Systat Version 9). Significantly more major volatile peaks were found in urine from the rut in contrast to both pre-rut, and post-rut samples ($p < 0.05$). Two major volatiles present in rut urine were p-cresol and geraniol; numerous other volatiles were present including 1-nonanol, 8-dodecenol, skatole, 1-octene, and alkane classes of compounds. One advantage of SPME headspace analysis compared with the conventional method of solvent extractions is that solvent impurities that can result in artifacts are greatly reduced. These data will serve as a baseline for further studies of solvent-based extractions of adult male moose urine. This research was funded by NIH/MFP Grant No. 5 T32 MH18882-13.

THE HONEYED MESSAGE OF MUSTH IN ASIAN ELEPHANTS, *Elephas maximus*

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Musth is a male phenomenon affecting many aspects of inter-elephant behavior. Young teenage male Asian elephants exhibit a short musth termed "moda" during which they release sweet-smelling exudates from their facial temporal gland. As males become older and increasingly socially mature, the temporal gland secretion (TGS) contains progressively more malodorous ketones together with a bicyclic ketal, frontalin. Behavioral observations of individual captive and wild elephants, concurrent with chemical analyses assessing musth status, demonstrated that moda males generally avoided both older, secreting musth males and samples of their collected TGS, whereas moda males or their sweet exudates elicited little response from older adults. Bioassays with captive elephants demonstrated while adult males were indifferent to the single compound, frontalin, young males were highly reactive, often exhibiting repulsion or avoidance. Frontalin assays with female elephants demonstrated that the frequency and types of chemosensory and behavioral responses were dependent on hormonal and reproductive status. Follicular-phase females often demonstrated mating-related behaviors subsequent to the high chemosensory responses to frontalin. Our data suggest that olfactory cues released by musth males affect inter-male behavior and that recognition of the ontogenic degree of musth in conspecifics prior to physical encounters is socially, reproductively and perhaps evolutionarily advantageous.

MECHANISMS FACILITATING SCENT OVER-MARKING IN FEMALE GOLDEN HAMSTERS

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Two separate mechanisms may be used to produce over-marks: (1) an odor-stimulated increase in the rate of marking and (2) targeting of the mark so that it overlaps a previously deposited scent mark. Although odor-stimulated marking has often been observed, no one has proven targeting. To do so, one must rule out the influence of animals preferring to be in or mark in particular physical locations (e.g., near walls of an arena). A series of experiments showed that although female hamsters do spend more time in the half of an arena where another female has marked with vaginal secretions and although they do deposit more marks in this part of the arena, their rate of vaginal marking was not increased by these odors. Over-marks did occur, but were they due to chance or to a targeting mechanism? We developed a new method to discriminate between targeting and chance occurrence. We recorded the exact position of every scent mark in one half of a symmetric arena and used this map of marks as a template for the position of "virtual marks" in the other half of the arena. Females then explored both halves of the arena; they over-marked the real marks more than the symmetrically placed "virtual marks". Thus, these results show that females target the marks of other females and must have specialized sensory-motor mechanisms to promote over-marking. This in turn suggests the importance of such marking in competition between females. Supported by NSF grant to R.E. Johnston

ODORS DETECTED BY GOLDEN HAMSTERS PRIOR TO TWO DAYS OF AGE ARE DIFFERENTIALLY UTILIZED DURING LATER DEVELOPMENT

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Although research with various rodent species has examined the development of olfactory function, little work has been done with golden hamsters (*Mesocricetus auratus*), one of the primary mammal models for studies of olfactory communication. Specifically, we are not aware of any studies that have investigated the ability of pups 1-2 days old to detect and remember specific odors. In the current study, mothers were scented with one of two artificial scents, vanillin or phenylethyl alcohol (PE), during the first 48 hours after delivery of a litter. The pups were then tested for their responses to these odors to determine if they detected and recalled the scent to which they had been exposed. At 12 days post-birth, pups raised by vanillin scented mothers spent more time investigating vanillin-scented plates than they did investigating PE-scented plates or a control plate. At 17 days post-birth, the pattern of results changed - the scent that the pup was raised with was treated more similarly to the control scent. However control pups raised without exposure to a foreign smell continued to show preferences or aversions to the various smells. This pattern of changes throughout development suggests that pups may change the type of responses they show toward odors associated with their mother as their own priorities change, e.g., at first to stay close to the mother and the nest and later to filter out this scent in exploration of other potentially useful ones such as those of a mate, enemy, or food. This research was supported by an NIMH grant to R.E. Johnston.

MAMMALIAN MODEL OF AGGRESSION AND SMELL

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In many species odors are involved in initiation and cessation of aggressive activities; however, it is unclear whether these two complex behavioral traits have an overlapping genetic basis. In our research we take advantage of the discovery that two inbred strains of mice, CBA (CBA/J) and NZB (NZB/B1NJ) serve as animal models for both extreme olfactory variation and for aggression. The phenotypes are sensitivity to androstene (5 α -androst-16-en-3-one) and the level of inter-male aggression. The androstene-sensitive and low-aggressive CBA strain and the androstene-anosmic and highly-aggressive NZB strain were used. In a classical genetic analysis we tested sensitivity to androstene in both types of F1 hybrids and in segregating F2 mice (n=104) using a "buried cookie test". Differences in sensitivity to AND between CBA and NZB mice were estimated to be at least 2,000-fold. Analysis of the results of AND sensitivity tests of the segregating F2 generation indicated the polygenic nature of this trait. The level of aggressiveness was quantified using a standard test with castrated male intruders serving as target mice. 93% of CBA mice did not attack the intruder. Of the NZB males, 88.2% exhibited high levels of aggression. Only 23% of CBA (female) x NZB (male) F1s were aggressive; however, 92% of the males from the reciprocal, NZB x CBA, cross revealed high levels of aggression. In the F2 generation, the level of sensitivity to AND is correlated with the level of male aggressiveness (r=0.78). A strong correlation between these two phenotypes in the segregating F2 generation suggests either linkage of genes controlling these behavioral traits or pleiotropy.

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PSYCHOPHYSICS OF ODOR DETECTION THRESHOLD IN A MODEL SYSTEM

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Psychophysical assessment of detection thresholds in the chemical senses is problematic because of the difficulties inherent in delivery of odorants in air. We developed an approach to assess detection thresholds that couples dilution of odor-saturated air with gas chromatographic evaluation of odor delivery. Stimuli are delivered through an olfactometer that mixes odor-saturated air with clean air. We differentially conditioned moths (*Manduca sexta*) to discriminate air from air mixed with odor. The detection threshold was assessed as the percent dilution at which stimuli were no longer discriminable. Detection thresholds for different odors were in the range of 10-20% odor saturation. We then quantified the delivery rate of odor using gas chromatography. Solid Phase MicroExtraction fibers (SPME) were placed both at the nozzle of the olfactometer and at the position of moth placement, which allowed us to estimate the amount of dispersion from the nozzle to the animal. Results indicate that thresholds of detectability for different odors are in the range of 0.5 to 10 nanograms per second depending on the odor. These estimates are significantly higher than those obtained for male moth sensitivity to female sex pheromone. This difference suggests that there may be a trade-off between sensitivity and tuning. More broadly tuned cells, which must respond to a very large variety of odors by way of a spatiotemporal "combinatorial" code, may be less sensitive than the much more narrowly tuned pheromone-sensitive cells. This work was supported by NIH-NCRR (9 R01 RR14166-06) to BHS and an award from the OSU Office of the Vice President for Research.

NOVEL RELEASE MECHANISM FOR A SEA LAMPREY SEX PHEROMONE

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A bile acid, 3 keto petromyzonol sulfate, has been identified as a male sea lamprey sex pheromone and shown to be released to attract conspecific females for spawning. We found that, unlike sex pheromones found in other fish species that are released through urine, the lamprey pheromone is released through the anterior portion of the body, more specifically through the gills. Lamprey holding water was collected from the anterior and posterior region of male sea lamprey using a bisected aquarium. Analyses of water washings using thin layer chromatography, enzyme-linked immunosorbent assay and mass spectrometry revealed that the pheromone existed in the washings from anterior regions at concentrations between 37.5 – 113.1 ng/ml, but was not detectable in washings from the posterior portion. In addition, a behavioral study using a two-choice maze confirmed that only washings from the anterior region could elicit behavioral response in ovulating females. Clearly, the pheromone is released from the anterior region of the fish. Further, to examine whether the gills are responsible for releasing the pheromone, an immunocytochemical study was performed using an antibody raised against the pheromone. In the gills, immunoreactive cells were observed in the glandular cells of spermiating males, the male individuals that actively release the pheromone. Taken together, we report that male sea lamprey employ a novel mechanism for releasing the sex pheromone and that the gills of the fish may play an important role in delivering the pheromone to the stream for reproduction. This study was supported by the Great Lakes Fishery Commission.

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EXPERIMENTAL EVIDENCE THAT 7, 12, 24-TRIHYDROXY-5-CHOLAN-3-ONE 24-SULFATE FUNCTIONS AS A SEA LAMPREY SEX PHEROMONE

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Recently it has been found that male sea lamprey (*Petromyzon marinus*) release a potent sex pheromone, 7, 12, 24-trihydroxy-5-cholan-3-one 24-sulfate (3-keto petromyzonol sulfate) upon spermiation. This pheromone appears to induce preference behavior and locomotion in ovulating females under controlled laboratory conditions. It has yet to be demonstrated that a synthetic copy of 3-keto petromyzonol sulfate stimulates the olfactory organ and induces characteristic behaviors in ovulating females. To provide conclusive evidence that synthetic 3-keto petromyzonol sulfate functions as a sex pheromone in a natural spawning environment, we first used electro-olfactograms (EOG) to determine the detection threshold and dose response curves of the synthetic pheromone. Then in a section of spawning stream we observed the behavioral response of ovulating females to the synthetic pheromone (at concentrations determined by EOG experiments). EOG results showed that the synthetic pheromone is detected at approximately 10⁻¹² M. When introduced into the spawning stream section, ovulating females swam to and stayed at the source of the synthetic pheromone. We conclude that synthetic 3-keto petromyzonol sulfate is able to function as a sex pheromone in a natural environment at concentrations that are likely to be encountered in the wild. To our knowledge this is the first vertebrate study to effectively demonstrate a synthetic pheromone compound in a natural environment. The Great Lakes Fishery Commission supported this study.

RELATING BEHAVIOR TO OLFACTION IN THE ROUND GOBY, *NEOGOBIOUS MELANOSTOMUS* (PERCIFORMES: GOBIIDAE).

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The round goby (*Neogobius melanostomus*) has established populations in all of the Laurentian Great Lakes following initial colonization in 1990 from the Ponto-Caspian region. We have shown that the peripheral olfactory organ in this fish contains a continuous sheet of flat olfactory epithelium that extends ventrocaudally from the anterior nostril to the accessory nasal sacs. Immunocytochemistry using antibodies against G_o and G_{olf} has revealed that G proteins are located on the apical surface of microvillar and ciliated olfactory receptor neurons, respectively. Using whole mounts, it has been shown that microvillar and ciliated olfactory receptor neurons are distributed evenly throughout the olfactory epithelium. For olfactory behavioural trials, dental impression material was used to plug the noses of the fish for sensory deprived negative controls. This procedure has also revealed that the round goby peripheral olfactory organ contains lachrymal and ethmoidal accessory nasal sacs. Gill ventilation rates were observed when male and female round gobies were subjected to the putative pheromones estrone and etiocholanolone at concentrations ranging from 10^{-8} to 10^{-12} M. Behavioural bioassays have shown that there was no significant change in ventilation rates between osmic and anosmic female round gobies. Ventilation rates in osmic male round gobies were significantly ($p < 0.05$) higher in response to concentrations of estrone at 10^{-8} M and 10^{-9} M than anosmic males, indicating that olfaction may play an important role in intraspecific communication. Supported by NSERC

THE EFFECT OF EXPOSURE TO DOMINANCE ODOR ON SOCIAL BEHAVIOR IN CRAYFISH, *ORCONECTES LITTORALIS*

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Agonistic interactions play an important role in the lives of many social species. These interactions are used to establish dominance relationships that determine which individuals will have access to the highest quality food, shelters or mates. Due to the benefits obtained from agonistic encounters, dominant individuals usually have a higher level of fitness compared to subordinates. A variety of factors influence the formation of hierarchical structures, including a crayfish's intrinsic aggressive state, an ability to physically dominate, previous agonistic experience, and dominance pheromones. Previously, we have shown that chemical communication via urine signals plays a significant role in recognition of aggressive state and may also carry information on dominance status. This study attempted to elucidate the behavioral effects of exposure to dominant odor without experiencing social interactions. Individuals were exposed to control, naïve, and dominant odors and the effects of these odors on their subsequent fighting behavior were analyzed. Since previous social odor experiences influence subsequent behavior, we hypothesize that previous odor exposure, mediated solely through chemical signals, may alter subsequent interactions. Support for this project was funded by the NSF Sensory Systems.

THE EFFECT OF ELEVATED CO₂ DETRITUS ON THE FORAGING DECISIONS OF CRAYFISH (*ORCONECTES VIRILIS*)

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Atmospheric CO₂ is expected to double in the next 50 years and research elucidating impacts to the biosphere are important. In lotic systems where leaf litter detritus comprises up to 99% of the carbon foundation of the food web, changes in detritus chemistry as a result of CO₂ changes may affect the behavior of organisms that feed on detritus. A y-maze was used to determine crayfish preference for detritus reared under ambient or elevated. Stimuli consisted of: 1) fresh detritus, 2) detritus leached for 24 hours, and 3) leachate from detritus. Within these preparations were three treatments with all pair-wise combinations. Behavioral parameters measured from videotapes. Crayfish preferred ambient stimulus over other stimuli tested and showed no preference in the elevated vs control when offered fresh detritus or leachate. These results demonstrate that crayfish can discriminate chemically between ambient and elevated detritus, that ambient detritus is preferred, and that crayfish are attracted by chemicals diffusing from the leaves. These changes in crayfish foraging decisions can affect the whole community. This research was funded by grants from the NSF IGERT Fellowship to JAA, NSF DEB to NCT, NSF DAB, NSF Sensory Systems, and a BGSU TIE grant to PAM, and the University of Michigan Biological Station.

INDUCED HOST PREFERENCE IN *SPODOPTERA LITTORALIS*

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Lepidopterous larvae have been shown to be able to acquire induced preferences to certain host plants through feeding experience. The physiological mechanisms behind induction are unknown but different theories have evolved, such as postingestive effects or different kinds of learning. One indication of learning would be a difference in the neuronal response-pattern between individuals of different nutritional history. Further, there are behavioral studies suggesting the survival of olfactory learning through metamorphosis in holometabolous insects. We seek to further penetrate this topic and in this study we a) investigate induction to three plants, cotton, oil seed rape and alfalfa, in larvae of the moth *Spodoptera littoralis*, and b) investigate whether differences in the neuronal activation patterns of the antennal lobe can be found in adult *S. littoralis* with different nutritional history. In the first part of the study, third to fourth instar larvae were tested in a two-choice olfactometer after rearing on one of the three plants. The larvae were induced to prefer the experienced food plant if it was a suitable food source. This was the case with cotton and alfalfa. Oil seed rape is a poor resource to *S. littoralis* and they were not induced to prefer this plant. In the second part, still in progress, we study the response-pattern in the antennal lobe of adult *S. littoralis*, reared on any of the three plants. We use calcium optical imaging to evaluate the neural activation. This work was supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS).

POLLINATION BY DECEPTION - CARRION MIMICRY IN THE DEAD HORSE ARUM

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The dead horse arum, *Helicodiceros muscivorus* mimics the odor and the structure of a carcass in order to attract carrion feeding insects, primarily blowflies. The attracted flies are then exploited as unrewarded pollinators. We have shown that the involuntary pollinators, by relying on odor cues, cannot separate the plant from a true food and oviposition site resource. Odor collections from the plant and from rotten meat contain the same active oligosulphide components, eliciting identical olfactory responses from the fly olfactory organ, the antenna. The chemical mimicry is accompanied by visual, tactile and thermal adaptations enforcing the mimicry. Participation in this system is detrimental to flies, thus a selective advantage for non-participating flies is expected. The longevity of the system likely relies on the fact that the flower mimics compounds typically found in any true resource of the flies, i. e. any fly failing to be attracted to the odorants will also fail to locate potential food and oviposition sites. The dead horse arum is a striking example of how a plant during evolution can acquire the ability to mimic an odor.

BITTERNESS INHIBITION USING THE BITTER COMPOUND UREA

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Previous cross-adaptation research from our lab indicated that the bitter compound urea may act as a general bitterness inhibitor. As part of a larger research project investigating bitter-bitter interactions we investigated the influence of urea on seven other bitter compounds: tetralone (iso-alpha acid mixture), denatonium benzoate, sucrose octaacetate, quinine-HCl, ranitidine, L-tryptophan, and L-phenylalanine. Psychometric curves were constructed for all compounds for each subject (n=17). All subjects rated bitterness on the Labeled Magnitude Scale. Four concentrations were chosen from the linear phase of the psychometric curve of each bitter compound. A weakly bitter concentration of urea was added. The bitter psychometric curves of the pure compounds were compared with the psychometric curve when urea was added. Weak concentrations of the bitter compounds were also added to four concentrations from the linear phase of urea's psychometric curve. Urea had no effect on the bitterness of tetralone. For the remaining bitter stimuli there were compound specific differences. However, urea had a suppressive effect on their bitterness, thereby supporting the hypothesis that urea, a bitter stimulus itself, can act as a bitter inhibitor in admixture without prior adaptation. Supported by NIH grant DC02995 to PASB

OLFACTORY DETECTABILITY OF SINGLE CHEMICALS AND MIXTURES

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We are exploring the olfactory and trigeminal detectability of binary chemical mixtures vis-à-vis detectability of the individual components. Here we study the olfactory detectability of butyl acetate and toluene, presented singly and in mixtures. Employing a three-alternative forced-choice procedure and an ascending concentration approach we have built concentration-response (i.e., psychometric) functions for the odor detectability of each chemical. Then, we selected certain detectability levels (e.g., 0.8) on a range from chance detection (0.0) to virtually perfect detection (1.0), and used the previously obtained functions to prepare mixtures of varying individual detectabilities (e.g., 0.2+0.6, 0.4+0.4, and 0.6+0.2) such that, if a rule of dose addition were to hold, all mixtures and each single chemical at the reference concentration should be equally detectable. Mixtures and their corresponding single components were tested under the same experiment, setting, and group of subjects. The results revealed that the mixtures are significantly less detectable than the single compounds, an indication of poor odor detection agonism. In comparison, trigeminal detection of mixtures of these same substances, via nasal pungency or eye irritation, shows a higher degree of agonism. Supported by grant number R01 DC 02741 from the NIDCD, NIH, and by the Center for Indoor Air Research.

SYSTEMATIC CHANGES IN THE TYPES OF ODOR INTERACTIONS ARE RELATED TO THE MOLECULAR STRUCTURE OF THE COMPONENTS

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Predicting what type of interaction will occur between odorants has not been possible because little attention has been given to the selection of odorants which could act as agonists or antagonists. On the basis of data from physiological and molecular biological studies which have defined the molecular features of odorants, namely, length of the hydrocarbon chain and position of the functional group in aliphatic aldehydes, that are required to stimulate receptor cells, the present study investigated the interactions of the C3 to C6 aldehydes with the C7 aldehyde heptanal. The interactions were conducted using a multi-channel computerized air dilution olfactometer. As predicted the C3 aldehyde suppressed heptanal in a non-reciprocal manner, a reciprocal suppressive interaction occurred between the C4 and C7 aldehydes and no interaction or additivity occurred between the C5, C6 and C7 molecules. It is proposed the non-reciprocal interaction occurred at the periphery with the smaller C3 molecule binding to but not activating the larger site of the C7 molecule. The larger molecule not being able to occupy the smaller site of propanal. The reciprocal concentration-dependent interaction with the C4 and C7 molecules is typical of intrabulbar interactions with the higher concentration odorant being the main suppressor. Additivity on the other hand indicates the C5, C6 and C7 molecules successfully activated the same receptor. These results appear to represent the first examples of applying structure-activity relationships to odor interactions with humans and obtaining systematic changes in the types of odor interactions that result.

DO SOMATOSENSORY TACTILE STIMULI INTERACT WITH TASTE AND AROMA SIGNALS TO MODULATE PERCEPTION?

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Flavour is defined as the combined perception of mouth-feel, texture, taste and aroma. Within the food industry, the ultimate aim is to understand how varying these flavour components affects flavour quality so that foods can be formulated to deliver maximum consumer acceptability when they are eaten. The perception of sweetness and flavour were studied in viscous solutions containing 5g/l sucrose, 100ppm iso-amyl acetate and varying concentrations of three hydrocolloid thickeners (guar gum, carrageenan and hydroxypropylmethyl cellulose (HPMC)). Zero-shear viscosity of the samples ranged from 1 – 5000 mPas. Perception of both sweetness and aroma were suppressed at thickener concentrations above c^* (critical coil overlap concentration). Sensory data for the three hydrocolloids was not adequately correlated with their concentration relative to c^* (c/c^* ratio), particularly above c^* . However, when perceptual data was plotted against the Kokini oral shear stress (τ), calculated from rheological measurements, data for the three hydrocolloids aligned to form a master-curve, enabling the prediction of flavour intensity in such systems. The fact that oral shear stress can be used to model sweetness and aroma perception supports the hypothesis that somatosensory tactile stimuli can interact with taste and aroma signals to modulate their perception. This work was funded by Firmenich SA Geneva.

THE COMPLEX AND INTRIGUING LIVES OF PIP2 WITH ION CHANNELS AND TRANSPORTERS.

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Phosphatidylinositol 4, 5-bisphosphate (PI4,5P2), the precursor of three signaling molecules, is itself also used to signal to membrane-associated proteins. Recently, ion transporters and channels have been discovered to be regulated by PI4,5P2, often via functional interactions with other modulators. Systems activated by PI4,5P2 include plasmalemmal calcium pumps (PMCA), cardiac sodium-calcium exchangers (NCX1), sodium-proton exchangers (NHE1-4), a sodium-magnesium exchanger, all inward rectifier potassium channels (KATP, IRK, GIRK and ROMK channels), epithelial sodium channels (ENAC), and some channels of the TRP family. Systems inhibited include rod-CNG channels, IP3 receptors, and several TRP family members. Presumably, local changes of the concentration of PI4,5P2 in the plasma membrane represent cell signals to these mechanisms, and our progress in studying the regulation of PI4,5P2 will be reported. In cardiac muscle, PI4,5P2 increases during trains of electrical stimulation, and it is decreased by diacylglycerol analogues and by free radical scavenging. In 6 different cell types, PI4,5P2 increases markedly with cell shrinkage, and these changes are not affected by numerous inhibitors of shrinkage-dependent signaling mechanisms. We are testing whether membrane tension and diacylglycerol may regulate the insertion of vesicles containing highly active lipid phosphatases.

INTRODUCTION: NEW INSIGHTS INTO PHOSPHOINOSITOL SIGNALING

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New insight into the complexity and ubiquity of phosphoinositol signaling in other cellular systems, coupled with increasing evidence for phosphoinositol signaling in chemosensory transduction, suggests revisiting this important, timely topic. Outside speakers will be invited to address two exciting developments in phosphoinositol signaling, the increasing number and diversity of calcium entry or TRP channels, and the growing evidence that not only the classical phosphoinositol turnover pathway involving activation of phospholipase C, but also membrane phosphoinositols themselves, often involving activation of phosphoinositide-3-OH kinase are key players in many cellular responses involving lipid second messengers. Local speakers will address how this insight is impacting our current understanding of chemosensory transduction in the main olfactory organ and in taste cells, and integrate with last year's symposium on chemosensory transduction in the vomeronasal organ.

DIVERSITY AND FUNCTIONS OF TRP CHANNEL SUBFAMILIES

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A large number of channel proteins with sequence homology to the *Drosophila* TRP (Transient Receptor Potential) cation channel has been identified in recent years on the basis of genome projects, and expression- and PCR-based cloning. Based on sequence analysis, the proteins can be divided into three groups. All mammalian isoforms are now named following a unified nomenclature as belonging to the TRPC (formerly STRPC), TRPM (formerly LTRPC), or TRPV (formerly OTRPC) group. Members of the TRP or canonical or classic subfamily are those most highly related to *Drosophila* TRP. Besides their structural similarity, they are all activated by receptor-mediated phospholipase C cascades. The TRPV subfamily is named based on the first mammalian member of the family, the vanilloid receptor (VR1). Members of the TRPV subfamily are assumed to be involved in osmo-, chemo- and mechano-sensory transduction, although their mechanism of activation is known only for the first and fourth isoforms. Melanostatin is the founding member of the TRPM subfamily and was described as a protein absent in malignant-transformed melanocytes. Despite having eight different members, the overall function of this group is still unclear and its proposed involvement in cell growth and differentiation is still very speculative. Classification of TRP channels initially based on sequence analysis is now verified by functional data obtained from proteins from *Drosophila*, *Caenorhabditis*, and mammals.

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3-PHOSPHOINOSITIDE SIGNALING IN OLFACTORY RECEPTOR CELLS

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Phosphatidylinositol 3-kinase (PI3K)-dependent signaling can be coupled to receptors for many different ligands and has been implicated in diverse cellular systems. PIP3, the primary product of PI3K activity in vivo, modulates a transduction-associated ion channel in lobster olfactory receptor cells. In acutely dissociated rat olfactory receptor cells, blocking PI3K enhances the response of the cells to complex, but not single odorants. PIP3 rescues the effect of blocking PI3K and inhibits odorant stimulation. The need to block phospholipase C (PLC) as well as PI3K in some cells suggests that 3-phosphoinositide signaling acts in concert with the canonical phosphoinositide turnover pathway. 3-phosphoinositides appear to act by modulating cyclic nucleotide signaling downstream of the receptor. Collectively, these findings implicate 3-phosphoinositide signaling in olfactory transduction. The ability of 3-phosphoinositides to inhibit cyclic nucleotide dependent excitation in an odorant-specific manner suggests that PI3K-dependent signaling has a functional role in odorant coding.

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PHOSPHOINOSITIDE SIGNALING IN TASTE CELLS

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Inositol 1,4,5 trisphosphate (IP3) has been implicated in the transduction of bitter compounds, artificial sweeteners, and recently, in umami taste. Of these pathways, the role of IP3 in bitter transduction is best understood. Bitter compounds bind to the T2R/TRB class of bitter taste receptors, which are coupled to the heterotrimeric G protein gustducin. Alpha-gustducin activates phosphodiesterase (PDE1A) to reduce intracellular cAMP, while its partners (3 13) activate PLC 2 to produce IP3 and diacylglycerol. Although the role of the reduced cAMP is not clear, the IP3 binds to receptors on the smooth endoplasmic reticulum to release Ca2+ from intracellular stores. In collaboration with R. Margolskee's laboratory, we have identified 2 downstream effectors of the bitter signaling cascade: the Type III IP3 receptor and a store operated channel, most likely Trp-T. Calcium imaging experiments with mudpuppy taste cells and GFP-labeled mouse taste cells expressing gustducin have shown that prolonged stimulation with the bitter stimulus denatonium elicits a transient increase in intracellular Ca2+ due to release from internal stores followed by a sustained influx of Ca2+. Treatment of taste cells with thapsigargin to deplete internal Ca2+ stores mimics the Ca2+ influx, suggesting that store-operated channels are present in taste cells and mediate the Ca2+ influx. Further experiments will be required to determine if Trp-T is the source of the Ca2+ influx.

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THE PREVALENCE OF ANDROSTENONE ANOSMIA MAY BE LOWER THAN PREVIOUSLY ESTIMATED

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Specific anosmia to androstenone has been examined in a variety of studies and is reported at about 30 % prevalence. This has typically been assessed by moderately strict objective measures, namely 2 or 3 alternative forced choice paradigms, with criteria set at 4 or 5 consecutive correct trials. This test, however, is optimized for type-two over type-one errors, i.e., optimized to not label a non-detector as detector at the cost of erroneously labeling some detectors as non-detectors. Such tests may have inflated the estimate of non-detection. To address this possibility we screened 31 subjects for detection of crystal androstenone using a relatively strict 4-repetition 3-alternative forced choice test. Subjects who were correct on 2 trials or fewer were labeled putative non-detectors. This screen yielded 5 subjects (16%) who were at 29% accuracy (chance = 33%). These 5 putative non-detectors were then subjected to a rigorous signal detection task. Either androstenone or a foil was presented 74 times (ISI = 45s) to blindfolded subjects who determined if an odor was present or not. Subjects obtained 58% mean accuracy (SD = 9). Four of the 5 subjects obtained a positive d' score (mean = .471) reflecting a trend towards detection (t(4) = 2.25, p = .09). In other words, 4 of 5 subjects labeled as non-detectors by standard screening methods may in fact be detectors. Additional subjects will be tested in order to address the possibility that the prevalence of specific anosmia to androstenone is in fact much lower than previously estimated. Funding: Searle Fellowship, SOSI, NIH-NIDCD

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DETECTION THRESHOLDS FOR PHENYL ETHYL ALCOHOL USING SERIAL DILUTIONS IN DIFFERENT SOLVENTS

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To determine odor detection thresholds subjects are presented with a series of odorant dilutions. The solvent used to dilute the odorant, how the odorant is presented and the methods used to define a threshold are important factors in determining threshold levels. Pierce and Doty (1996) reported differences in detection thresholds for phenyl ethyl alcohol (PEA) when they used different solvents. In this study we used gas chromatography (GC) to further investigate the effect of solvent on PEA odor detection thresholds. We used a single ascending method and serial dilutions of PEA in four different solvents; liquid paraffin (LP), mineral oil (MO), propylene glycol (PG) and dipropylene glycol (DPG) to determine the PEA thresholds for 31 adult subjects. For each solvent we prepared 8 serial log step dilutions (1 to 8) with corresponding liquid PEA concentrations of 6.3×10^{-1} to 6.3×10^{-8} (vol/vol). We found that the average threshold dilutions for PEA in LP (6.4) and PEA in MO (5.8) were lower than for PEA in PG (4.2) and DPG (4.2). We used GC to measure both the liquid and gas PEA concentrations for the dilution steps prepared with LP and PG. Although the liquid threshold concentrations of PEA in LP (6.4) and PG (4.2) were different, there was no difference in the PEA gas concentrations. Partition coefficients (K) for PEA in LP (8.73×10^{-6}) and in PG (2.74×10^{-7}) were determined from gas/liquid concentration ratios. These results demonstrate the importance of measuring the gas concentration of the odorant stimuli when comparing odor detection thresholds obtained using different solvents or delivery methods.

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DETECTION OF GLUTARALDEHYDE IN WATER: CHANGING SENSITIVITY AND SPECIFIC ANOSMIASchmidt R.¹, Cain W.S.¹ ¹*Surgery, University of California, San Diego, La Jolla, CA*

A study of thirty adults (15 males, 15 females, ages 18 to 40 years), screened to have normal olfaction (normosmia), measured detection of the flavor of glutaraldehyde, a biocide that could occur in disinfected potable water. Over the range of interest, flavor derived from olfactory stimulation. Fourteen subjects failed to detect the glutaraldehyde in the first of four sessions of testing. Eight of the 14 (seven males, one female) continued to exhibit the anosmia throughout testing. The other six (one male, five females) began to detect the material in session two and exhibited increasing sensitivity over sessions two to four. Their sensitivity never reached that of the 16 subjects who evinced no anosmia and who also improved their performance over sessions. The combined group of 22 could detect 17 ppm. Less thorough testing would have yielded much higher values than obtained here, with potentially negative consequences for water quality. Specific anosmia for this dialdehyde has precedence in anosmia for various monoaldehydes, most notably isobutyraldehyde. The positive influence of experience with a material on detection has been found previously, most intriguingly those of Wysocki and colleagues, who showed that experience could differentially induce sensitivity to the odorant androstenone and suggested that the phenomenon might occur for other compounds. Glutaraldehyde appears to be one, perhaps of many. Supported by the Union Carbide Corp., a division of Dow Chemical Co.

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OLFACTORY DISCRIMINATION OF FATTY ACIDS IN RATS WITH LARGE BILATERAL LESIONS OF THE OLFACTORY BULBSBisulco S.¹, Slotnick B.¹ ¹*Department of Psychology, American University, Washington, DC*

Rats were trained in an 8-channel Knosys olfactometer to discriminate between acetic acid (S+) and caproic acid (S-) and between acetic acid (S+) and propionic acid (S-). To insure that discrimination was not based on intensity cues, we varied the concentrations of each odor over a wide range within test sessions. Rats were retested on these tasks and also on a +carvone (S+) vs -carvone (S-) task after they received large bilateral bulbar lesions that removed and extended well beyond the fatty acid responsive areas identified in electrophysiological and optical imaging studies and removed the anterior fatty acid sites identified in 2-DG studies. All rats achieved criterion performance on all tasks, although on average, experimental rats made slightly but not significantly more errors than did controls. We conclude that removal of bulbar areas identified as responsive to the odors of short-chain fatty acids are essentially without effect on the ability to use qualitative differences in discriminating between dissimilar (C2 vs C6) and similar (C2 vs C3) acid odors or between the enantiomers of carvone.

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ORAL FAT EXPOSURE AUGMENTS THE "SECOND MEAL" EFFECT IN HUMANSMattes R.D.¹ ¹*Foods and Nutrition, Purdue University, West Lafayette, IN*

Oral exposure to dietary fat augments the postprandial rise of serum triacylglycerol (TAG) due, in part, to an influence on lipid absorption. One mechanism may involve release of lipid stored in the lacteals from the previous meal. This was explored in a repeated measures design study with 16 healthy adults. Participants ingested 80g of almonds (~53%en oleic, 14%en linoleic) at 2200h as the last eating occasion of the evening prior to each testing day. For testing, participants reported to the lab after an overnight fast and three blood samples were drawn over 20 minutes followed by ingestion of 50g capsules of safflower oil (~13%en oleic, 77%en linoleic) with 500ml of water in 10 minutes. After another blood draw, they received 10s oral exposures to cream cheese on a cracker, or cracker only every 5 minutes for 60 minutes and every 15 minutes for 60 additional minutes. Blood samples were drawn every 2 minutes for 24 minutes and then at minutes 30, 60, 90, 120, 240, 360, 480 and 560. The samples were analyzed for TAG, oleic acid and linoleic acid. Biphasic patterns of TAG were noted in all but one participant with the initial peak occurring at 12-30 minutes and the second peak generally present at about 240 minutes after oral stimulation. The oleic acid concentration in peak 1 associated with cracker and cream cheese stimulation was significantly greater than the level in peak 2 or the concentration in peak 1 after cracker only stimulation. The oleic/linoleic ratio in peak 1 was significantly greater than the ratio in peak 2. These data document a second-meal effect (i.e., release of stored lipid) with oral stimulation that is greater for cream cheese plus cracker compared to the fat-free cracker alone. Supported by PHS grant #R01-DK45294.

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SENSORY MEASUREMENT OF DYNAMIC FLAVOR PERCEPTION IN ICE CREAM WITH DIFFERENT FAT LEVELS AND FLAVORINGSBom Frøst M.¹, Heymann H.², Bredie W.¹, Dijksterhuis G.B.¹, Martens M.¹ ¹*Dept. Dairy and Food Science, Royal Veterinary and Agricultural University, Frederiksberg, Denmark;* ²*Food Science Dept., University of Missouri, Columbia, Columbia, MO*

Intro: Flavor compounds vary in physicochemical properties and, therefore behave differently in foods with different fat content. The objective was to investigate differences in dynamic flavor perception in a realistic food system, and relate them to a range of molecular descriptors for flavor compounds. Ice creams with different fat levels (3, 6, and 12% milk fat) and flavoring (-ionone (berry), -nonalactone (coconut), isopentyl acetate (banana), vanillin (vanilla)) were examined. Approximately equi-intense concentrations (in 12% fat) were selected. Samples were analyzed with descriptive analysis and Time-Intensity, evaluating perceived melt rate and flavor intensity (trained panel N=12, 3 replicates). Data were analyzed by ANOVA, Principal Component Analysis (PCA) and Partial Least Squares Regression (PLSR). Results: Descriptive analysis showed large differences in sensory properties for ice creams with different fat levels, and significant effects of fat level on flavor intensity for all four compounds. PCA of Time-Intensity showed faster perceived melt rates, increases and decreases in dynamic flavor perception with lower fat levels. Individual flavor compounds were affected differently by changes in fat level. By PLSR increase and decrease rates of dynamic flavor perception were reliably modeled to a number of molecular descriptors. Grant support: Danish Dairy Research Foundation (Danish Dairy Board) and Danish Government. Flavor donation: Haarmann & Reimer GmbH.

301 Poster Assessment of Discrimination and Sensitivity**DETECTING MALINGERERS WITH PSYCHOPHYSICAL TESTING**

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²*Psychology, University of Colorado, Boulder, CO*

We tested the ability of the maximum-likelihood adaptive staircase psychophysical procedure to discriminate true anosmics from malingers. Twenty subjects were tested: 7 normals, 6 anosmics, and 6 cheaters. The experimenter did not know which subject belonged to which group. The PI instructed each subject to do the test as instructed by the experimenter (normals), to try to convince the experimenter with their smelling behavior that they were unable to smell (cheaters), or to do the test as asked without telling the experimenter that they were unable to smell (anosmics). All but one cheater returned for testing to confirm that they were in fact normal. Left and right nostrils were tested separately. Subjects were paid for their participation. Detection thresholds for butyl alcohol were determined with a 2AFC maximum-likelihood adaptive staircase procedure. The procedure estimates the threshold after each trial and chooses the concentration of the next stimulus that is closest to the estimated threshold. Each subject was presented with 20 trials. Two measures, threshold stimulus concentration and probability of being correct over the 20 trials, can discriminate the cheaters from true anosmics, but not perfectly. Discriminability $d(a)$ was 1.1 and 1.3 respectively. Cheaters had significantly higher detection thresholds than anosmics; cheaters also had detection performance significantly worse than chance (0.29) whereas anosmics performed at chance (0.5). Supported by NIH grant PO1 DC00244

302 Poster Assessment of Discrimination and Sensitivity**CORRELATIONS BETWEEN INTRANASAL ANATOMY AND HUMAN OLFACTION**

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The objective of this study was to identify regions in the nasal cavity which determine olfactory function. To this end, a study was performed in 28 healthy male subjects (age range 22 to 57 years). All subjects met inclusion criteria which was ascertained by a thorough history. Olfactory dysfunction, chronic or acute sinus disease, previous trauma, head surgery, steroidal medication, allergies or neurological disorders were considered exclusion criteria. Informed consent was obtained in writing. Nasal flow was measured by anterior rhinomanometry. Olfactometry was performed by testing odor identification, odor discrimination and butanol odor thresholds with the "Sniffin' Sticks"™ test kit. Cranial MRI scans (T1, T2, T2 3d and flair sequences) were obtained immediately following olfactometry. The nasal cavity was divided into 22 areas, 11 at either side. Volumes of these areas were measured and correlated to the olfactory test results. Significant correlations between nasal volumes and test results of olfactory function were found for the upper meatus, in front of and in the olfactory cleft. The present results indicate two nasal volume segments important for interindividual differences of odor threshold in healthy subjects: (1) the region in the upper meatus below the cribriform plate and (2) the nasal valve region (inferior meatus of the anterior nasal cavity). These results are of significance for treatment of respiratory hyposmia. In a clinical context, the regions related to olfactory function should be considered when planning and performing intranasal surgery as septoplasty and inferior turbinectomy. This study was supported by the University of Cologne.

303 Poster Assessment of Discrimination and Sensitivity**INTEGRATED STORAGE AND COMPOUND MANAGEMENT SYSTEM TO SUPPORT HIGH THROUGHPUT DISCOVERY**

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The use of high throughput chemistry coupled with high throughput screening methods is finding its way into the discovery process of new flavor and fragrance molecules. These techniques, which were developed over the last ten years by the pharmaceutical industry, introduce problems of storing, safely handling, and tracking of a large number of volatile molecules and associated safety information. We will describe a web-based system implemented at Senomyx that allows the researcher to obtain important safety information using a combination of online resources, informatics tools and an Oracle database of chemical information. This system also allows us to track the physical location of over 8000 bulk chemicals and to search for these molecules using physical and calculated properties such as molecular weight, chemical structure, clogP, and vapor pressure. The integration of informatics with a well-managed storage system facilitates the use of these molecules for research and product development.

304 Poster Biologically Relevant Odors in Humans**DETECTION THRESHOLDS FOR 4,16-ANDROSTADIEN-3-ONE**

Olsson M.J.¹, Lundstrom J.N.¹, Hicks A.S.¹ ¹*Psychology, Uppsala University, Uppsala, Sweden*

Several studies have investigated the psychological and physiological effects of the putative pheromone 4,16-androstadien-3-one (androstadienone). However, no attempts, to our knowledge, have been made to measure the detection thresholds for this substance. Twenty-two women and 20 men participated in a threshold test (3-alternative forced-choice) in the concentration range of zero to 3000 micromolar. The substance was dissolved in propylene glycol and presented in 180ml polypropylene squeeze bottles. Preliminary data suggest that the sensitivity distribution across individuals is not unimodal. A few "supersmellers" were identified that could reliably detect the weakest concentration (76 micromolar). When supersmellers were excluded, the group-mean threshold approximated 300 micromolar and men and women did not differ in thresholds. The results are discussed in relation to current pheromone research. (HSFR: F0868)

THE SMELL OF EMOTION: OLFACTORY COMMUNICATION OF EMOTION IN HUMANSChen D.¹, McClintock M.K.¹ ¹*Psychology Department and Institute for Mind and Biology, University of Chicago, Chicago, IL*

Emotions are often considered the motivator of behavior and the basis of social interactions. Whilst it is well established that humans communicate emotions through facial, vocal, and verbal expressions, little is known about their olfactory communication. In contrast, olfactory communication of emotions, particularly of sexual arousal and fear, is well documented in animals from invertebrates to vertebrates. We will present here evidence that humans do communicate emotions through olfactory signals. Prior work established that, when given a choice, women could identify odors of people when they were happy and odors of men when they were afraid. We replicated and extended this finding in a study in which underarm odors from men and women were collected when they were under neutral and emotional states of happiness, fear, and sexual arousal. The odors were in turn evaluated by a group of judges on odor discrimination and identification tasks. We found that a small percentage of people identified above chance the target emotions on a forced choice task, but that a majority of men and women discriminated above chance between odors from neutral and emotional states from the same individual. This finding was further replicated in a second study in which heterosexual partners served as both donors and judges. Together, these studies support the hypothesis that emotional state in humans can be conveyed through sweat. This work was supported by funding from the Sense of Smell Institute, and by a NIMH Merit Award to MKM.

PSYCHOLOGICAL EFFECTS OF SUBTHRESHOLD EXPOSURE OF 4,16-ANDROSTADIEN-3-ONE ON WOMENLundström J.N.¹, Olsson M.J.¹ ¹*Psychology, Uppsala University, Uppsala, Sweden*

The aim of the current study was to investigate the replicability of mood effects of a subthreshold concentration of the putative human pheromone 4,16-androstadien-3-one (androstadienone) in women by comparing a new experiment (Experiment 1) with a previously reported experiment (Experiment 2; Lundström, et al., 2001). In Experiment 1, 38 women participated in a double blind, counterbalanced, between-groups experiment. Participants were exposed to either a solution consisting of 250 micromolar of androstadienone in mineral oil, masked with 1% Eugenol, or to a solution of mineral oil and Eugenol only. Mood was measured before and 20min after exposure onset. Among the nine mood variables, there was a significant (positive) change only in participants' feeling of being focused ($p = .01$). In Experiment 2, 37 women participated in an experiment almost identical to Experiment 1. Differences were that Experiment 2 utilized a within-groups design, the participants were tested on day 12-14 in their menstrual cycle, and propylene glycol was used as the solvent. Again, a significant and positive change of the participants' feeling of being focused was observed ($p = .004$). In both experiments, sensory detection of androstadienone was rigorously controlled for. It could therefore be ruled out that the observed effects were mediated by conscious experience. The test-retest reliability between the two experiments and across the nine mood variables was assessed with correlational analysis ($r = .77$, $p = .01$). Altogether, the results suggest that mood effects of androstadienone in women are weak but persistent. (HSFR: F0868)

ANDROSTADIENONE AFFECTS COURTSHIP-LIKE BEHAVIORS IN WOMENWilson P.¹, Haviland-Jones J.², Wysocki C.J.³, Warrenburg S.¹, Christensen C.¹ ¹*Research & Development, International Flavors & Fragrances, Union Beach, NJ*; ²*Psychology, Rutgers, The State University of New Jersey, Piscataway, NJ*; ³*Monell Chemical Senses Center, Philadelphia, PA*

Missing from the growing body of research on the effects of the putative pheromone, androstadienone (AND), is behavioral data. To investigate such behavioral effects, 80 couples were exposed to one of four conditions: clove oil alone, fragrance alone (RD01-014-IFF, Union Beach, NJ), clove oil with AND or fragrance with AND. Opposite-sex dyads, strangers to each other, interacted doing a social task. Their behavior was videotaped, coded and analyzed and mood questionnaires were completed. Women exposed to AND increased a set of behaviors that include "courtship" or flirtatious body language, while they reduced signals indicative of nervousness ($F(1) = 7.225$, $p = 0.009$). They reported a decrease in depression ($F(1) = 8.876$, $p = 0.004$). The AND with fragrance group showed lower fear/anxiety ($F(1) = 8.876$, $p = 0.004$). Ability to smell AND did not affect these results. This behavioral change without awareness of odor complements earlier research on sexually dimorphic neuroendocrine function and decreased negative mood in females. There is no evidence that attraction to the partner was changed. The only direct effect on males was an increase in happy/interest for males partnered with women exposed to AND ($F(1) = 4.3256$, $p = 0.043$). Therefore, AND has significant direct effects on human behavior and emotions. However, while AND may affect the likelihood of signaling a partner, it does not affect attraction to a specific partner, at least not in this context.

PHYSIOLOGICAL AND PSYCHOLOGICAL EFFECTS OF TWO PUTATIVE HUMAN PHEROMONESBrown W.¹, Johnson B.², Mainland J.³, Bremner E.³, Tsutsui T.¹, Moguel S.¹, Bensafi M.³, Sobel N.³ ¹*Psychology, University of California, Berkeley, CA*; ²*Bioengineering, University of California, Berkeley, CA*; ³*Neuroscience, University of California, Berkeley, CA*

The putative human pheromones androstadienone (AND) and estratetraenol (EST) are sex steroid derivatives present in human sweat. Brain imaging has shown these compounds affect brain activity in a sex specific manner. We therefore hypothesized that AND and EST may induce sexually dimorphic psychological and physiological effects reflecting these alterations in brain activity. In a within subjects design we evaluated the effects of AND, EST and a control substance (baking powder) on mood and physiology. 26 subjects (12m/14f) had 5 minutes of baseline recorded for all variables before compound exposure. They then watched a neutral nature video for 40 minutes while data were recorded. This procedure was repeated with a different compound for each subject on three separate days. All analyses were completed on change scores. As predicted, the two compounds affected several psychological and physiological measures in a sex-specific manner reflecting brain activation. For example, a double-dissociation was evident in GSR whereby AND induced greater increases in men, and EST induced greater increases in women, while the control induced equal changes in both sexes ($F(2,26) = 3.2$, $p < .05$). AND also increased positive moods (e.g., amused; content) in men but not in women ($P < .01$). These dissociations suggest that AND and EST may be acting as human pheromones to uniquely alter physiology and mood. Funding: Searle Fellowship

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CROSS-CULTURAL INVESTIGATION OF BODY ODOR

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28 subjects from Japan and non-Japanese countries were asked to wear a T-shirt for 4 consecutive nights. All subjects used the same soap and shampoo for their daily hygiene, took a bath or a shower once a day, and ate a similar Japanese diet. On the fifth day the T-shirts were presented to 30 Japanese who were asked to rate the odor of the T-shirts. They were asked to judge whether the owner of the T-shirt was a woman or a man, Japanese or non-Japanese, and to rate the pleasantness and the intensity of the body odor. The results demonstrated that the Japanese subjects were able to identify the gender of the T-shirt owner, regardless of whether the owner belonged to their own or to a different culture. They were also able to correctly decide whether the T-shirt was worn by a Japanese or not. The odor of the non-Japanese T-shirts was perceived as more intense than the odor of the Japanese T-shirts. No difference was found in the pleasantness perception of Japanese and non-Japanese T-shirts. These findings indicate that a) the ability to discriminate between the smell of a woman and a man is not restricted to one's own culture, b) Japanese and non-Japanese might differ in the intensity of their body odor, c) the body odor of subjects from foreign countries is not perceived as less pleasant when the diet is similar to one's own culture.

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HUMAN AXILLARY ODORS FORMED BY ENDOGENOUS BACTERIA

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The axillae support high densities of aerobic bacteria of the Micrococcae family (*Staph. species*) and diphtheroids (*Corynebacterium* [C.] and *Brevibacteria* genera). We have demonstrated that characteristic axillary odors (C6-C11 acids) result from base hydrolysis or incubation of *C. lipophilicus* (CLC) with water-soluble apocrine secretion molecules. Here we evaluated axillary bacteria from male donors for production of 3-methyl-2-hexenoic acid (3M2H), other acids and volatile steroids. Apocrine secretions were obtained by dilute epinephrine injection. Experiment 1 used separate water- and organic-soluble apocrine secretion molecules incubated for 24h with 4 different bacteria. All bacteria liberated 3M2H from the water-soluble fraction. Little or no androstenol or androstenone were found, which is consistent with previous results demonstrating that volatile steroids play a minor role in axillary odor. CLC produced the largest amounts of 3M2H. In Experiment 2 secretions were not separated. Acids up to C12 were identified and quantified: CLC produced the greatest levels of 3M2H; *Staph. species* produced the largest levels of C2-C5 acids, including isovaleric acid. These data suggest that CLC, rather than other bacteria, are more proficient in producing characteristic axillary odors using incubation times that normally exist in the axillae (~24h between showers) and that organic acids are the principle odorants. Our data suggest that axillary organic acids are likely involved in pheromonal affects. Supported by grants from NIH (DC 01072) and Haarmann and Reimer.

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ANALYSIS OF DOSE-RESPONSE FUNCTIONS IN THE ANTENNAL LOBE OF THE MOTH SPODOPTERA LITTORALIS

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The olfactory system of all organisms has to identify a varying number of different odours. In addition the system should be able to recognise concentration changes without losing the qualitative discrimination power. At the level of sensory neurons, numerous studies have demonstrated the physiological dose-response characteristics. However, only limited information is available about concentration coding in higher brain centra. In an earlier calcium imaging study of the antennal lobes of the moth *Spodoptera littoralis* we found that the glomerular activity was distributed but odour specific. The glomerular representations were often overlapping in that each glomerulus was activated by several odorants and each odorant activated more than one glomerulus (Carlsson et al., in press). In the present study we wanted to investigate how the glomerular representations were affected by changes in stimulus concentration. We measured neuronal activity indirectly by optical recordings of calcium concentration changes. The outlines of glomeruli were made visible after the calcium recordings by staining with a membrane bound dye and calcium responses could be correlated with anatomical maps of glomeruli. The number of activated glomeruli increased at elevated stimulus concentrations, most likely reflecting a recruitment of ORNs with higher threshold levels. However, dose-response curves for a given odorant were not always parallel in activated glomeruli. Furthermore, different odorants showed different dose-response characteristics within the same glomerulus.

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SENSORY PROCESSING OF ENVIRONMENTAL-CO₂ INFORMATION IN THE MOTH NERVOUS SYSTEM

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Insects can sense the CO₂ level in the air around them, and they are thought to use that information in vital tasks such as locating food sources. It is uncertain, however, how that information is processed by the CNS. In order to address this issue, we are studying the highly developed CO₂-sensing system of the moth *Manduca sexta*. We have characterized the physiology of CO₂-receptor cells located in the labial palps by means of extracellular recordings, and of CO₂-responding neurons in the CNS using intracellular techniques. In addition, we have used staining techniques to study the central projections of CO₂-receptor cells and the arborization pattern and projections of CNS neurons that respond to CO₂. Our results suggest that: (1) the receptor cells in the labial palps of female *Manduca* are specialized for the measurement of the surrounding CO₂ level; (2) those cells can encode step increases and decreases in CO₂ concentration, as well as the background CO₂ level; (3) their axons project bilaterally to a specific glomerulus in the antennal lobe (AL) of the brain, confirming previous findings; (4) neurons in the AL receive and process CO₂ information from the labial palps, confirming that the AL is the primary site for processing CO₂ information; and (5) CO₂-responding AL neurons can encode increases in CO₂, but their ability to encode decreases in CO₂ appears to be limited. Our findings reinforce the idea that information about environmental CO₂ plays important roles in the biology of moths and motivate further investigations of this remarkable chemosensory subsystem. Supported by NIDCD grant R01-02751.

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REPRESENTATION OF PHEROMONE BLENDS BY PROJECTION NEURONS IN HELIOTHINE MOTHS

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The manageable complexity of the male moth macroglomerular complex (MGC), a substructure composed of a few glomeruli within the antennal lobe (AL), combined with an extensive knowledge of the pheromonal odor ligands represented therein makes the MGC an experimentally favorable model system in which to address some general, yet unanswered questions of odor coding. We sought to quantify the ratio of dendritic arborizations of multiglomerular MGC projection neurons (PNs) with respect to the pheromone blend ratio that elicited an optimal response. Characterization of both blend and component specific neurons also allowed us to evaluate whether the PN population represented odor quality through either an assembly code (with neurons restricted to single glomeruli) or a more continuous code (with numerous neurons arborizing in >1 glomeruli). Processing modifies the final representation of information departing the AL, though the nature of this processing remains elusive. A "third-party" odor to which blend-sensitive PNs do not respond when applied separately, was added to the optimal pheromone blend. We predicted that such changes in odor quality would alter the sensitivity or response to the optimal blend. Across-glomerular interactions under conditions of changing odor quality were examined in this way. Finally, the predictions derived from this study will be tested in animals where the AL structure is experimentally modified by transplantation of the imaginal antennal disk across species. Supported by NIH 1 R55 DC04443-01 to C.L.

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SYNCHRONY AND SPATIOTEMPORAL ODOR CODES IN ANTENNAL LOBES OF THE MOTH MANDUCA SEXTA

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Odor codes in the insect antennal lobe (AL) or vertebrate olfactory bulb are spatially and temporally distributed across a reasonably large subset of neurons. To explore how changing patterns of responses code for odor, we implanted multichannel probes into the moth AL and recorded ensemble responses to 20 100ms pulses for 15 different alcohols and ketones that varied in carbon chain length and functional group position. Odor-evoked responses of cells within the ensemble showed consistent patterns of excitation and inhibition to single odors. Changes of odorant molecular structure did not necessarily produce change in the spatial response pattern. But it frequently produced significant change in the pattern of excitation and inhibition observed in individual cells of the ensemble. Thus, there was a temporally distinct response for each odor across cells in the ensemble. Using principle components analysis we classified temporal response features and patterns of synchrony of the responses to odor stimulation. Analyses of these patterns using general linear modeling revealed that variation in patterns of synchronous activity were indeed attributable to the molecular structure of odors. These results support our previous behavioral analysis that showed; 1) odor space has a dimensional nature based on odotopic variation; 2) moths discriminate differences in odorants that vary, in some cases, by single a carbon atom. This fine discrimination is achieved through changing patterns of synchrony among cells in a largely spatially overlapped response. This work was supported by NIH-NCRR (9 R01 RR14166-06) to BHS & (1 R03 DC05535-01) to KCD.

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ANALYSIS OF ODOR SELECTIVITY IN THE MOTH ANTENNAL LOBE USING NEURAL-ENSEMBLE RECORDING

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A growing body of evidence suggests that the chemical identity of a given odorant is encoded in the specific combination of glomeruli activated or inhibited by that odorant. This is especially true for sex-pheromone detection in insects, but it can be argued that the principles underlying pheromone processing may be very different from those for more 'general' odorants. We are therefore using neural-ensemble recording methods to compare the spatiotemporal patterns of activity evoked by both pheromonal and non-pheromonal odors in the antennal lobe (AL) of the moth. We are developing methods that allow us to distinguish different types of neurons in the ensembles by combining intracellular and ensemble recordings. Our results indicate that, like the pheromone-responsive ensembles we have studied previously, the ensemble response to each general odorant exhibits a unique pattern across output neurons. Some neurons are highly selective for one or a few odorants that belong to the same chemical class, while others are broadly responsive to odorants in different classes. Ensemble activity is concentration dependent, and neurons that display excitatory responses to one odorant are often inhibited by other odorants. Neurons having similar response profiles also tend to fire in synchrony. In summary, general odorants evoke overlapping patterns of activity across ensembles of AL output neurons that are very heterogeneous in their tuning properties. Moreover, precise temporal interactions among specific subsets of neurons in these ensembles may provide a mechanism for encoding specific stimulus features. Supported by a grant from NIH-NIDCD (DC02751).

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RELATIONSHIP BETWEEN RECEPTOR NEURON INPUT AND INTRINSIC OPTICAL SIGNALS IN THE MOUSE OLFACTORY BULB

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We imaged odorant-evoked receptor neuron input to mouse olfactory bulb glomeruli using calcium-sensitive dyes (Wachowiak and Cohen, Neuron 32:723-735). In the same preparations, we recorded intrinsic optical signals using 630 nm reflected light (Rubin and Katz, Neuron 23:499-511). At low concentrations, odorants evoked input to a few glomeruli. Intrinsic signals appeared as restricted foci and corresponded closely to the glomeruli activated by receptor neuron input. Threshold concentrations for eliciting calcium and intrinsic optical signals were similar. Increasing odorant concentration recruited input to widespread glomeruli and increased the spatial distribution of intrinsic signals, such that intrinsic signals were apparent in regions showing no detectable receptor input. The increased distribution of the intrinsic signals was accompanied by a decrease in their spatial definition. Spatially filtering the intrinsic signal in order to isolate single glomeruli resulted in fewer apparent glomeruli than could be resolved by imaging receptor neuron input with calcium dye. Calcium imaging also revealed glomerulus- and odorant-specific differences in the temporal dynamics of receptor neuron input. The corresponding intrinsic signals showed little or none of these dynamics. These results suggest that, while intrinsic optical signals may be strongly driven by receptor neuron input, they also reflect postsynaptic olfactory bulb activity which may be more spatially homogeneous. These two components are not easily separated on the basis of spatial or temporal features. Supported by NIH DC00378 and NS08437.

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REGULATION OF RECEPTOR NEURON INPUT TO THE MOUSE OLFACTORY BULB MEDIATED BY SUPPRESSION OF PRESYNAPTIC CALCIUM INFLUX

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Multiple lines of evidence suggest that receptor neuron input to the mammalian olfactory bulb is negatively regulated by presynaptic inhibition. We have characterized this inhibition directly by imaging Ca^{2+} influx into olfactory nerve (ON) presynaptic terminals using a mouse olfactory bulb slice preparation. Single shocks to the ON layer evoked rapid (~20 msec rise-time) fluorescence increases followed by a slow, monophasic decay. This response was largely blocked by ω -conotoxin GVIA, an N-type Ca^{2+} channel blocker. Paired ON shocks revealed a strong and long-lasting suppression of presynaptic Ca^{2+} influx, with 40 - 60% suppression at 200 ms interstimulus intervals and a recovery time of ~1 sec. Blocking activation of postsynaptic olfactory bulb neurons with APV/CNQX reduced this suppression. The GABA_B agonist, baclofen, strongly inhibited presynaptic Ca^{2+} influx, while the GABA_B antagonist, CGP55845, reduced paired-pulse suppression without affecting the response to the conditioning pulse. The D_2 agonist quinpirole weakly suppressed Ca^{2+} influx when presented alone, but showed a significant suppression of the response when co-applied with CGP55845. These results are similar to those reported for turtle ORNs using the same Ca^{2+} imaging technique, and support earlier electrophysiological studies in mammals suggesting that both GABA_B and D_2 receptors mediate presynaptic inhibition of olfactory input to the olfactory bulb. Supported by NIH DC00378 (MW) and DC00347 & DC36940 (MTS).

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DISTINCT ACTIVITY PATTERNS EVOKED BY ACTIVATION OF MITRAL/TUFTED CELL AND CENTRIFUGAL FIBER INPUTS TO MAIN OLFACTORY BULB (MOB) GRANULE CELLS.

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Granule cells (GCs) receive excitatory input from mitral/tufted cells as well as from centrifugal fibers (CFF) inputs originating, in part, from primary olfactory cortical areas. Here, we compared activity patterns evoked by CFF activation to those elicited by antidromic activation of mitral/tufted cells via lateral olfactory tract (LOT) stimulation in mouse MOB slices. Activity was monitored using extracellular field potentials (FPs) and voltage sensitive dye (RH414) optical signals (OS) recorded with a photodiode array. CFF stimulation robustly increased OSs in the GC layer (GCL) (n=6/6 slices); with a delay of 5-6 msec, moderate activity was subsequently observed in the external plexiform layer (EPL) (n=6/6), and in some cases (n=2/6), weak activity in the glomerular layer (GL). LOT shocks, by contrast, produced strong OSs in the EPL (n=5/5), accompanied simultaneously by moderate and weak OSs, respectively, in the GCL (n=2/5) and GL (n=2/5). CFF shocks produced a negative FP in the GCL and a corresponding positivity in the EPL. CFF and LOT-evoked OSs/FPs were completely abolished by CNQX, TTX or lowering extracellular Ca , whereas AP5 reduced them by 18-35%. These results indicate that CFF- and LOT-evoked activity in the MOB primarily represents postsynaptic responses in GCs. CFF inputs robustly activate GC somata/proximal dendrites, while mitral/tufted cell inputs are focused on GC distal dendrites in the EPL. GC responses to both synaptic inputs are mediated by ionotropic glutamate receptors, with dominant AMPA, and moderate NMDA, receptor-mediated components. Support: PHS grants DC03195 & DC00347.

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A CODE FOR COMPLEX OBJECTS: EMERGING PRINCIPLES FOR NATURAL ODOR REPRESENTATION IN THE MOUSE BRAIN

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The olfactory system detects small differences in the composition of natural odorants, made up of hundreds of molecules. Odorous quality is hypothetically represented by a combinatorial code -activation of distinct, but overlapping subsets of olfactory receptors resulting in activation of a distinct subset of glomeruli in the olfactory bulb. Here we show that modification of a single gene (the K gene of the major histocompatibility locus), which results in a subtle change in the odiferous quality of urine, causes a small, but significant change in the glomerular activation pattern. Moreover, we show that spatial activity patterns contain enough information to discriminate among behaviorally discriminable natural odors. In addition, the magnitude of disparity between urine-evoked glomerular activation patterns is predictive of the extent of genetic difference among the donors and the receiver's ability to discriminate. These data show that the combinatorial code applies to natural odors.

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COMPUTATIONAL TOOLS FOR MAPPING THE GLOMERULAR LAYER OF THE OLFACTORY BULB.

Salcedo E.¹, Schaefer M.L.¹, Restrepo D.¹ ¹*Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO*

The sweet smell of chocolate and other complex odors are initially processed and coded in the mammalian main olfactory bulb. Responses to different odors generate distinct glomerular activity patterns, or "odor maps." These maps are thought to form the neural basis for the discrimination of different odors. Thus, an important metric for the investigation of olfactory coding is the precise position of an individual glomerulus within an olfactory bulb. To accurately compare results from different laboratories, this metric must be standardized. As previously reported, we have developed a Glomerular Analysis Program that provides the computational tools necessary to map the location of an individual glomerulus to within a statistically defined domain. We have updated this program to include different definitions of the point of origin. Additionally, we have added the ability to generate 2D maps of the glomerular sheet using equal area projections. Finally, we have added the ability to visualize the olfactory bulb as a 3D composite of glomeruli that can be rotated at will. In a companion application, ToMatrix, we have developed several algorithms to compare multiple 2D contour maps using a variety of statistical analyses. With this software package, we hope to standardize the methods for collecting and comparing information on odor maps. This work was funded by NIDCD DC00566 and by NS07083.

DISTRIBUTION OF CORRELATED ACTIVITY IN THE OLFACTORY BULB OF RATS FROM SIMULTANEOUS MULTIELECTRODE RECORDINGSLehmkuhle M.J.¹, Normann R.A.¹, Maynard E.M.¹ ¹Bioengineering, University of Utah, Salt Lake City, UT

The goal of this study was to investigate the network properties of populations of mitral/tufted cells in response to enantiomer odor pairs using microelectrode arrays. Specifically, the distribution of correlated firing rates as a function of electrode separation and enantiomer pair used were observed. Two Utah Electrode Arrays, each with as many as 16 electrodes spaced 400µm apart, were inserted into the mitral cell layer of both hemispheres of the dorsal aspect of olfactory bulb of five anesthetized rats. Four enantiomer pairs of the same concentration were delivered to rats through a digitally controlled olfactant delivery system. Simultaneous recordings from up to 32 microelectrodes per experiment recorded single- and multi-unit activity from an average of 25 mitral/tufted cells. The average number of electrodes with activity per experiment was 14 or 43.8% of available electrodes. The average number of single- and multi-units per electrode with activity was 1.8. Trial-by-trial correlations in the firing rates were slightly higher for distant electrodes; electrodes near each other were uncorrelated. The mean correlation of pairs of units separated by 0, 400, and 800µm were -0.02, 0.15, and 0.26, respectively. Microelectrode arrays, simultaneously recording action potentials from a large number of mitral cells in the rat olfactory bulb, offer a unique means of investigating the ways ensembles of neurons process temporally correlated olfactory information. NIH grant 1R43DC04261-01, 01-00053-5000-54900-44500

LATERAL HYPOTHALAMUS AND AMYGDALA MODULATE TASTE RESPONSES OF PARABRACHIAL NEURONSLi C.¹, Cho Y.K.¹, Smith D.V.¹ ¹Anatomy & Neurobiology, University of Maryland, Baltimore, Baltimore, MD

Neuroanatomical studies show reciprocal connections between the parabrachial nuclei (PbN) and several forebrain nuclei, including the central nucleus of the amygdala (CeA) and the lateral hypothalamus (LH). Our previous studies have shown that electrical stimulation of the ipsilateral LH antidromically activates 84% of PbN taste cells whereas 63% are antidromically invaded from the ipsilateral CeA. Electrical stimulation of the ipsilateral LH and CeA also orthodromically activates 11% and 25% of PbN taste cells, respectively. Thus, in addition to their influence on the nucleus of the solitary tract, these forebrain nuclei modulate PbN taste cells as well. In the present study, we further examined whether electrical and chemical stimulation of the LH and CeA enhance spontaneous activity or taste responses of PbN cells. Glass micropipettes were glued to concentric bipolar stimulating electrodes and implanted into the LH and CeA. After confirming that each PbN cell was orthodromically activated from the LH and/or CeA, a brief subthreshold electrical stimulation (100 Hz, 0.2 ms, 15s) or DL-homocysteic acid (DLH, 10 mM) was delivered to the forebrain sites. Taste solutions were 32 mM NaCl, sucrose, and quinine hydrochloride and 3.2 mM citric acid. Electrical stimulation of the LH or CeA increased the responsiveness to taste stimulation of these PbN neurons. Microinjection of DLH into the LH or CeA increased the spontaneous activity of orthodromically activated PbN taste cells. These results further demonstrate that both the LH and CeA modulate the activity of brainstem taste cells. Supported by NIDCD DC00066 (DVS).

NEUROBIOLOGY OF THE RAT INFERIOR SALIVATORY NUCLEUSKim M.¹, Cheigo Jr. D.J.², Bradley R.M.² ¹Nursing, Chonnam University Medical School, Gwangju, Korea; ²Biologic & Materials Science, University of Michigan, Ann Arbor, MI

Saliva plays a pivotal role in taste mechanisms, and stimulation of taste receptors initiates secretion. Despite this interdependence little is known about gustatory-salivatory reflexes. The parasympathetic secretomotor neurons that control salivary glands are located in the inferior and superior salivatory nuclei and are closely apposed to the nucleus of the solitary tract (NST), the taste relay nucleus in the brainstem. However, knowledge of the biophysical and morphological properties of the salivatory neurons and their synaptic connections with the NST is lacking. In initial approaches to learn about morphological characteristics, we analyzed soma and dendrite properties of neurons in the inferior salivatory nucleus (ISN). Neurons were labeled by retrograde transport of Fluorogold applied to the glossopharyngeal nerve. Horizontal, 100 µm brainstem sections were subsequently viewed in a confocal microscope and a stacked series of 1µm images was collected. Sixty individual neurons were then traced and analyzed using Neurolucida image analysis software. As a population, the morphometric characteristics of the soma and dendritic trees had a unimodal distribution. However, when neurons in the caudal, intermediate and rostral thirds of the ISN were compared, we found that the dendrites of neurons in these three regions extended at different orientation angles and had significantly different lengths and number of dendritic segments. Since the ISN controls both the parotid and von Ebner salivary glands, these differences in neuronal morphology may relate to the different secretory characteristics of these glands. Supported by NIDCD grants DC-00288 to RMB.

CONVERGENCE OF FOREBRAIN INFLUENCES ON TASTE NEURONS OF THE SOLITARY NUCLEUSCho Y.K.¹, Li C.¹, Smith D.V.¹ ¹Anatomy & Neurobiology, University of Maryland, Baltimore, Baltimore, MD

Both the lateral hypothalamus (LH) and the central nucleus of the amygdala (CeA) are involved in the regulation of ingestive behavior. These nuclei send descending axons to the nucleus of the solitary tract (NST). We investigated the effect of LH and CeA stimulation on the activity of NST gustatory neurons. Bipolar concentric stimulating electrodes were implanted in the LH and CeA bilaterally and the activity of 215 NST neurons was recorded. More than half (113/215) the taste-responsive cells in the NST were modulated by LH and/or CeA stimulation. The LH influenced more cells (101 vs. 64) than the CeA; 52 cells were modulated by both sites. Contralateral stimulation of the LH and/or CeA was more often effective (144 responses) than ipsilateral (74). Modulatory effects were mostly excitatory (102 cells); 11 cells were inhibited, mostly by ipsilateral LH. A subset of cells (n = 25) was examined for the effects of microinjection of DL-homocysteic acid (DLH), a glutamate receptor agonist, into the LH and/or CeA. The effects of electrical stimulation were mimicked by DLH, indicating that cell somata in and around the stimulating sites are responsible for these effects. Other cells (n = 25) were tested for the effects of electrical stimulation of forebrain sites on the responses to taste stimuli (32 mM sucrose, NaCl and QHCl, and 3.2 mM citric acid). Taste responses were enhanced by the excitatory influence of the LH and/or CeA. These data demonstrate that modulatory influences from the LH and CeA converge onto cells of the gustatory NST and modulate their taste responses. Supported by NINDS DC00066 (DVS).

MODULATION OF PONTINE TASTE ACTIVITY BY CENTRIFUGAL INPUTS UNDER TASTE

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Previous studies have suggested that taste responses of neurons in the parabrachial nucleus (PBN), the second taste relay in the rodents, are modulated under different internal environmental conditions. For a further studying of such modulation, we examined the effects of electrical stimulation of the gustatory cortex (GC) and central nucleus of the amygdala (CeA) on PBN taste response in control rats and rats exhibiting conditioned taste aversion (CTA) to 0.1M NaCl. Single unit responses were recorded from the PBN in Wistar male rats deeply anaesthetized with urethane. The taste stimuli were 0.5M sucrose, 0.1M NaCl, 0.01M HCl, 0.02M quinine HCl, 0.1M KCl and 0.1M NaCl+10⁻⁴M Amiloride. We have so far recorded 29 units in the control and 31 units in the experimental rats. Among them, the only amiloride-sensitive NaCl-best units (N=13) showed larger responses to NaCl in the experimental rats than in control rats. GC stimulation enhanced responses in 20.7% and inhibited 13.8% of PBN units in experimental rats, while in 19.4% and 29.0%, respectively, in control rats. CeA stimulation enhanced responses in 12.9% and inhibited in 41.9% in experimental rats, while it only inhibited in 51.7% in control rats. These results suggests that PBN taste responses are modulated by centrifugal influences under taste aversion learning. Supported by Grants-in-Aid for scientific research (Nos. 11557135 to TY) from the MEXT of Japan, and by JSPS-RFTF97L00906.

VARIABILITY OF TASTE RESPONSE MAGNITUDE IN THE NUCLEUS OF THE SOLITARY TRACT WITH STIMULUS REPETITION

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Taste-responsive cells are often classified by the stimulus that evokes the most robust response, i.e. the best stimulus. To test whether this characteristic is reliable, taste stimuli were presented repeatedly and electrophysiological responses were recorded from single cells in the nucleus of the solitary tract of anesthetized rats. Stimuli consisted of NaCl (.1 M), sucrose (.5 M), quinine HCl (.01 M) and HCl (.01 M), each followed by a water rinse. Blocks of the 4 tastants were repeated for as long as the cell remained isolated. Nineteen cells have been recorded with between 8 and 27 repetitions of each stimulus. Fifteen cells showed a consistent best stimulus across all blocks of trials, though responses varied widely in absolute magnitude. Responses >10 sps varied on average by 38% +/- 4% SEM. There was a significant correlation between response magnitude and the range of response (r=.85, p<.01). Breadth of tuning also varied significantly across blocks of trials. In 4 cells, the best stimulus changed across repetitions, varying between NaCl and HCl. In all cells, responses to non-best stimuli across repetitions varied to the extent that no information about stimulus identity could be determined solely from response magnitude. These results imply that for most cells relative response magnitude can discriminate between a single stimulus and all others. Other cells may utilize other coding mechanisms, e.g. temporal coding, to discriminate among taste stimuli. Supported by NSF grant BNS-0077965 to PMD and NIH grant EY9314 to JDV.

EFFECTS OF GLOSSOPHARYNGEAL ANESTHESIA ON TASTE RESPONSES IN THE NUCLEUS OF THE SOLITARY TRACT OF THE RAT

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Various studies in humans and rodents have suggested that the input from the facial nerve inhibits responses produced by the glossopharyngeal (GP) nerve in the nucleus of the solitary tract (NTS). To investigate this possible interaction, single-unit neural responses to gustatory stimuli (NaCl (1.0 M), sucrose (0.5 M), QHCl (0.01 M) and HCl (0.01 M) were recorded in the NTS prior, during and post anesthetization of the IXth nerve in urethane-anesthetized rats. Anesthetization of the IXth nerve was accomplished by local application of 2% lidocaine to the nerve. This reversible removal of IXth nerve input to the NTS resulted in stimulus-specific changes in the response profiles of NTS neurons. Specifically, the responses to sucrose were attenuated on average by about 2/3 in all 8 sucrose best units and responses to NaCl were on average more than doubled in 10 of 32 units. These changes in neural responses were restored to baseline levels upon the recovery of the IXth nerve from anesthesia. Only 2 units showed a decrease in response to all tastants following GP anesthesia. Interestingly, GP anesthesia did not significantly affect responses to quinine. These findings suggest that the IXth nerve input to the NTS generally converges with that of other taste nerves forming complex inhibitory/excitatory interactions. Supported by NIH grant 1F31DC05100-01A1 to CGR and NSF grant BNS-0077965 to PMD.

GURMARIN SUPPRESSION OF SUCROSE RESPONSES IN RAT SOLITARY NUCLEUS NEURONS

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In rats and mice, the peptide gurmarin partially inhibits chorda tympani (CT) responses evoked by sweet stimuli, but not by salts, acids and bitter compounds. Moreover, gurmarin inhibits responses to sucrose in some sugar-best CT fibers in the mouse, leaving others unaffected. This differential effect is somewhat analogous to the influence of amiloride on the neural processing of salt information, which is restricted to sucrose- and NaCl-best neurons. Therefore, we examined the effect of gurmarin on the responses of single neurons in the nucleus of the solitary tract (NST) of the rat to determine whether gurmarin sensitivity is differentially distributed across neuron types. Baseline responses to 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl and 0.01 M QHCl were initially recorded from each cell and a sucrose concentration series (0.01-1.0 M) in a subset of cells. Gurmarin (10 µg/ml, 2-4 ml) was then applied to the tongue and palate. After 10 min, the stimuli were reapplied. The response to 0.5 M sucrose was reduced by 60% in all sucrose-best neurons and by 33% in all NaCl- and HCl-best cells, except for a small subset of NaCl- and HCl-best neurons in which there was no effect. There was no effect of gurmarin on responses to NaCl, HCl or QHCl. The response to the sucrose concentration series was similarly shifted in all neuron types. These data suggest that information from the receptors sensitive to gurmarin converge onto several cell types in the rat brainstem and is not restricted to a single cell type. Supported by NIDCD DC00353 (DVS).

AN ARTIFICIAL NEURAL NETWORK MODEL OF TASTE INFORMATION PROCESSINGLemon C.H.¹, Smith D.V.¹ ¹*Anatomy & Neurobiology, University of Maryland, Baltimore, Baltimore, MD*

We have devised an artificial neural network (ANN) based on principles derived from taste neurobiology. An ANN application programming interface was developed and used to implement both an artificial taste (AT) and a pattern recognition (PR) network. The AT consisted of three layers of feed-forward information processing nodes. The activation function of each node within a layer incorporated predictors derived from the tuning profiles of chorda tympani (CT), solitary nucleus (NST) or parabrachial (PbN) taste neurons. Connection strength between nodes in consecutive layers was determined by multiple regression analysis of responses recorded from CT, NST and PbN neurons, which revealed how much variance in NST or PbN profiles could be explained by input from cell types in the CT or NST, respectively. A simulated taste input resulted in a pattern of activation across the initial AT layer that was propagated forward and processed by each subsequent layer. The final output of the AT served as input to the PR, which used an error backpropagation algorithm to converge. The AT produced unique output patterns for each input. The amount of information contained within each pattern was sufficient for discrimination, as the PR was capable of distinguishing among AT outputs produced by different simulated taste inputs. Multidimensional scaling revealed the organization of AT output patterns to be similar to those observed for PbN neurons in response to sweet salty, sour and bitter compounds. This system constitutes the base of an ANN system that may be used to simulate information processing in the gustatory system. Supported by NIDCD DC00353 (DVS)

IONIC CONTROL OF SWEET TASTE QUALITYHaywood K.A.¹, Giraud D.A.², Birch G.G.¹ ¹*School of Food Biosciences, The University of Reading, Reading, Berkshire, United Kingdom*; ²*ENSABANA, University of Burgundy, Dijon, Bourgogne, France*

Apparent specific volumes are known to be related to taste quality and can be manipulated by addition of salts that interact strongly with water structure. Optimisation of sweet taste quality using this type of approach is now a target for taste chemists. Although there are many food salts that can contribute to this end they are limited by their own intrinsic taste properties and choice of an appropriate salt for taste control depends on a combination of strong water interaction and bland intrinsic taste. The first of these conditions depends on multiplicity of ionic charge or more exactly charge density. The second depends on absence of effective sapophores. The only permitted food salt that appears to meet these requirements is trisodium phosphate, which has a negative apparent specific volume ($< -0.1 \text{ cm}^3 \text{ g}^{-1}$) and a correspondingly strong negative apparent specific isentropic compressibility. It therefore has the capacity to lower the apparent specific volumes of permitted sweetener solutions, and is unique in this capacity, as a permitted food salt. Preliminary sensory tests, with intense sweetener solutions, show that trisodium phosphate can lower the intensity and persistence of sweetness and improve quality. Demonstration of this effect in commercial sweetened products is contingent on phosphate being low or absent in their formulation. However, a sensory test with a commercial cola product showed a significantly higher hedonic score (3.92) for the product with the addition of 50 ppm trisodium phosphate than without (3.34). This illustrates the role of water-interaction salts in taste quality modulation.

ELECTRICAL STIMULATION OF THE PBN ELICITS INGESTIVE OROMOTOR BEHAVIORS IN CONSCIOUS RATS: A TOPOGRAPHIC ANALYSIS.King M.S.¹, Dykes R.E.¹, King C.T.² ¹*Biology, Stetson University, Deland, FL*; ²*Psychology, Stetson University, DeLand, FL*

The primary taste responsive region in the pons is in the caudal parabrachial nucleus (PBN), specifically the central medial (CM) and ventral lateral (VL) subnuclei surrounding the 'waist' (W) region (Norgren and Pfaffmann, '75; Halsell and Travers, '97). Since these PBN regions also project to brainstem oromotor centers (Karimnamazi and Travers, '98), they may play a role in the initiation of oromotor behaviors following activation of taste pathways. This possibility was investigated by electrically stimulating the PBN via implanted stainless steel wire electrodes in conscious rats and counting oromotor responses. The application of current (0.4 msec, 50 Hz, 50-200 μA) into CM, VL or W caused a 7-fold increase in ingestive oromotor behaviors over pre-stimulation levels ($n=7$, $p<.01$). Oromotor behaviors only increased during the stimulation period, rapidly returning to baseline levels when current application was terminated. Stimulation just rostral to W, but still within VL, also increased the number of ingestive oromotor behaviors ($n=6$, $p<.01$). This effect persisted for 1 min following stimulation. Stimulation of other PBN sites ($n=5$) as well as outside of the PBN ($n=4$) did not increase oromotor behaviors over pre-stimulation levels. The data suggest that neurons in the CM and VL subnuclei of the PBN, both surrounding and just rostral to W, are involved in the initiation of ingestive oromotor behaviors in rats. Our current research focuses on whether oromotor behaviors increase following PBN stimulation due to activation of motor or sensory pathways. [Support: NSF (IBN 0090641)].

WHERE ARE THE CRITICAL REGIONS FOR BRAZZEIN'S SWEET TASTE? HUMAN PSYCHOPHYSICAL AND MONKEY ELECTROPHYSIOLOGICAL RESPONSES TO BRAZZEIN MUTANTSJin Z.¹, Danilova V.¹, Markley J.², Hellekant G.¹ ¹*AHABS, University of Wisconsin-Madison, Madison, WI*; ²*Biochemistry, University of Wisconsin-Madison, Madison, WI*

Brazzein is a small, heat stable, intensely sweet protein. Based on des-pGlu1-brazzein, the wild type minor form of brazzein (WT), twenty-five brazzein mutants have been designed and produced. One-dimensional ¹H NMR spectroscopy showed all mutants were folded correctly. We conducted human psychophysical and monkey electrophysiological experiments with these brazzein mutants. A taste panel of 9 human subjects evaluated the sweetness of WT brazzein and its 25 mutants at the concentration of 100 mg/l with water as a control. Compared with WT, 3 brazzein mutants had increased sweetness, 17 mutants had decreased sweetness, while 5 mutants were no different from the wild form. In the rhesus monkey, responses of single chorda tympani taste fibers were recorded during tongue stimulation with the same stimuli. WT brazzein elicited good responses in taste fibers predominantly responsive to sweeteners (S fibers). The responses of the S fibers to 5 mutants were increased, to 13 mutants decreased and to 7 mutants were no different from the WT. The correlation coefficient between the human and monkey data was 0.77 with $p\text{-value} < 0.001$. The good correlation between the two approaches clearly showed that monkey serves as a good model for evaluation of new sweeteners. Our results provide an insight into the chemical and structural requirements for the sweet taste of brazzein as well as a systematic approach to improve the sweet potency of this low-caloric natural sweetener.

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Sweet Taste

GYMNEMA SYLVESTRE SWEET-BLOCKING EFFICACY ON TONGUE TIP VS. WHOLE MOUTHDelwiche J.F.¹, Beilstein C.² ¹*Food Science, Ohio State, Columbus, OH;* ²*Psychology, OSU, Columbus, OH*

This study investigated differences in the sweet-blocking efficacy of *Gymnema sylvestre* (GS) for different sweeteners (acesulfame K, sucrose, glucose and Na saccharin) when presented to the tongue tip or the whole mouth. Participants assessed the stimuli following pretreatment with GS tea, rating sweeteners solutions (and water) using tongue tip or whole mouth stimulation in different sessions. They indicated if each stimulus was sweet or not sweet, and gave a sureness judgment, from which the R-index, a signal detection measurement, was calculated. Repeated measures ANOVA on R-index values of 12 subjects indicate that there is no significant difference in the sweetness between sweeteners ($p=0.20$), that they are more readily perceived as sweet with stimulation of the whole mouth than with the tongue tip ($p=0.0004$), and that GS blocks sweeteners to different extents ($p=0.02$). All stimuli but saccharin were perceived as being sweeter when presented whole mouth, which showed no significant difference between stimulation conditions (Scheffe's test, $p=0.24$). Only the whole mouth presentations of sucrose, glucose and aspartame were significantly above chance performance ($\alpha=0.05$, Bi & O'Mahony, *J. Sens. Studies*, 1995), indicating that GS blocked sweetness completely for tongue tip stimulation, but not whole mouth stimulation for most sweeteners. Whether this is due simply to the phenomenon of spatial summation (whereby perceived intensity increases with increasing area of stimulation), or due to differences in physiological mechanisms between the tongue tip and the rest of the mouth, remains to be determined. *Self-funded project*

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Sweet Taste

MIMICRY OF SUCROSE TASTE WITH NON-CALORIC SWEETENERSD'Angelo L.L.¹, King G.A.¹, Dubois G.E.¹ ¹*Technical Division, The Coca-Cola Company, Atlanta, GA*

Sucrose exhibits a clean well-rounded sweet taste, while non-caloric sweeteners do not. However, non-caloric sweetener blends have been found to more closely reproduce sucrose taste. We conducted cross-adaptation experiments on sucrose, aspartame, saccharin, ace-K, cyclamate and sucralose for the purpose of determining the compositions of blends that reproduce sucrose taste as closely as possible. The cross-adaptation results showed similarity between saccharin and ace-K, and difference between aspartame and saccharin. Cyclamate is unique among all the sweeteners tested. It does not cross-adapt significantly with any of the sweeteners tested, which suggests that it may activate a unique subtype of sweetener receptor.

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Sweet Taste

DISCRIMINATION OF GLUCOSE AND FRUCTOSE AFTER ADAPTATION BUT NOT BEFORETharp C.D.¹, Breslin P.A.¹ ¹*Sensory, Monell Chemical Senses Center, Philadelphia, PA*

We have previously demonstrated that glucose and fructose are indistinguishable at multiple intensity levels when their concentrations are adjusted appropriately. Using the same whole-mouth, forced-choice taste matching paradigm we have reestablished that 200 mM fructose is indistinguishable from ~400 mM glucose. These same subjects were then adapted to the 200 mM fructose standard and also used this solution as their inter-stimulus rinse. For all subjects, discriminability between glucose and fructose increased. This may be taken as evidence that the two saccharides stimulate separate receptors; however, the possibility exists that non-sweet cues (e.g., viscosity, side-tastes, etc.) become more apparent when sweetness is reduced. Thus, the experiment was repeated under conditions of added 100 ppm lactisole (the sweet taste inhibitor) to all solutions. Glucose-fructose discrimination remained at chance. Despite the monogeusia of glucose and fructose, they appear to stimulate sweet taste via non-identical transduction mechanisms. This work was supported by NIH DC 02995 to PASB.

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Sweet Taste

GENERALIZATION OF A CONDITIONED AVERSION TO LICK-CONTINGENT ELECTRICAL STIMULATION OF THE NUCLEUS OF THE SOLITARY TRACT TO A NATURAL TASTEHallock R.¹, Di Lorenzo P.M.¹ ¹*Psychology, Binghamton University, Binghamton, NY*

Previous work from our lab has shown that electrical stimulation of the nucleus of the solitary tract (NTS) can produce a taste-like sensation in the awake, behaving rat. These results showed that rats could acquire an aversion to lick-contingent electrical stimulation in the NTS designed to mimic the temporal pattern of the electrophysiological response to sucrose (sucrose simulation pattern). Here, a single electrode was implanted unilaterally in the taste portion of the NTS, and rats were screened for reactivity to a quinine simulation pattern of stimulation. An aversion was established by pairing the sucrose simulation pattern of electrical stimulation with an IP injection of LiCl. We then tested for generalization to the 4 basic tastants (QHCl, NaCl, HCl, and sucrose). The tastants and water were presented in 1-min trials, and the number of licks was recorded. Results showed that all rats successfully formed a conditioned aversion to the sucrose simulation pattern. With one exception, rats that showed a generalized aversion to a natural taste specifically avoided sucrose. Extinction to lick-contingent electrical stimulation also extinguished the sucrose aversion. These results imply that it is possible to mimic some aspects of the perception of sucrose with a unique temporal pattern of electrical stimulation. Supported by NSF grant BNS-0077965 to P.M.D.

DIFFERENTIAL EFFECTS OF ESTROGEN ON LICKING RATES AND INGESTION OF SUCROSE AND NaCl SOLUTIONSCurtis K.S.¹, Davis L.M.¹, Therrien K.¹, Contreras R.J.¹ ¹*Psychology, Florida State University, Tallahassee, FL*

Estrogen affects food and fluid intake by rats. The goal of this experiment was to determine whether the effect of estrogen on ingestion is mediated, in part, by changes in taste responses to sucrose and NaCl. Female rats (n=8), trained to consume fluids rapidly during 10-s trials, were ovariectomized and allowed to recover for 1 wk. Rats then were given estradiol benzoate (EB; 10 µg) or the oil vehicle (VEH) and tested for taste responses to sucrose solutions (0.025M-0.4M) in 10-s trials, and subsequently for overnight intake of water and 0.025M sucrose. The same animals then were given EB or VEH and tested for taste responses to NaCl solutions (0.028M-0.05M NaCl in 0.05M sucrose) in 10-s trials, and subsequently for overnight intake of water and 0.5M NaCl in 0.05M sucrose. EB-treated rats licked significantly less sucrose overall during 10-s trials compared to VEH-treated rats ($F(1,28)=5.21$, $p<0.5$), but consumed comparable amounts of sucrose during overnight access. In contrast, EB-treated rats licked NaCl solutions at rates comparable to those of VEH-treated rats during 10-s trials, but consumed significantly more NaCl during overnight access (21.0 ± 5.2 ml vs. 12.9 ± 2.8 ml; $p<0.05$). These results show that EB has differential effects on taste responses to sucrose and NaCl solutions as well as on overnight intakes of sucrose and NaCl solutions. However, differences in rates of licking do not correspond to differences in ingestion; thus, the effect of estrogen on taste responses may not underlie observed changes in ingestion. Supported by NIH Grant DC 04785

USE OF AN OPERANT SIGNAL DETECTION TASK TO ASSESS SUCROSE SENSITIVITY IN INBRED MOUSE STRAINS DIFFERING IN SUGAR PREFERENCEEylam S.¹, Spector A.C.¹ ¹*Department of Psychology, Univ. of Florida, Gainesville, FL*

It has been proposed that the genetic variation in sweetener intake and preference in inbred mice is attributable in part to the *Sac* locus. Recently, this locus was shown to encode for the T1R3, a receptor that interacts with certain sweet-tasting ligands. To date, behavioral assays of genetic variations in responsiveness to sweeteners have been based primarily on the 2-bottle intake test. We attempted to complement such findings by measuring concentration-dependent responsiveness to sucrose using a signal detection task based on operant conditioning methodology in 4 strains of mice differing in sugar preference: C57BL/6J (high), SWR/J (medium), and DBA/2J and 129P3/J (low). Water-restricted mice (n=11/strain) were trained, in a specially designed gustometer, to lick from one side spout in response to a sucrose stimulus sample and to lick from another side spout in response to a water stimulus sample. Correct responses were reinforced with water through these side spouts. Mice from all 4 strains were successfully trained in this paradigm; the average hit rate of the 4 strains for the 2 highest concentrations after 3 weeks of testing was 78.2 – 84.4%, while the average false alarm rate was very low (~16%). Now that we have demonstrated that stimulus control of responding is achievable in this explicit signal detection task with these mouse strains, we are in the process of measuring sucrose taste detection thresholds to test whether the previously reported preference variation is related to taste sensitivity differences. Supported by NIDCD R01-DC04574.

GUSTATORY RESPONSES TO POLYCOSE IN FOUR SPECIES OF NONHUMAN PRIMATESLaska M.¹, Kohlmann S.¹, Hernandez-Salazar L.T.², Rodriguez-Luna E.² ¹*Department of Medical Psychology, University of Munich, Munich, Germany;* ²*Instituto de Neuro-Etologia, Universidad Veracruzana, Xalapa, Mexico*

The taste responses of six squirrel monkeys, five pigtail macaques, four olive baboons and four spider monkeys to polycose, a starch-derived polysaccharide, were assessed in two-bottle preference tests of brief duration (2 min). In experiment 1, the monkeys were given the choice between tap water and defined concentrations of polycose dissolved in tap water. In experiment 2, the animals were given the choice between polycose and sucrose, fructose, glucose, lactose and maltose presented in equimolar concentrations of 100 and 200 mM, respectively. The animals were found to significantly prefer concentrations of polycose as low as 10 mM (pigtail macaques), 30 mM (olive baboons and spider monkeys) and 60 mM (squirrel monkeys) over tap water. Relative taste preferences were stable across the concentrations tested and indicate an order of relative effectiveness (sucrose > polycose ≥ maltose) in squirrel monkeys, spider monkeys and olive baboons which is similar to the order of relative sweetness in humans. The pigtail macaques, however, displayed an order of relative effectiveness (maltose > polycose ≥ sucrose) which differs markedly from that found in the other primate species tested and is similar to relative taste preferences found in rodents such as rats. Both the high sensitivity of the pigtail macaques for polycose and their vivid predilection for this polysaccharide and its disaccharide constituent maltose suggest that *Macaca nemestrina*, unlike other primates, but like rodents, may have specialized taste receptors for starch.

POLYMORPHISMS OF THE MOUSE TAS1R3 GENE ARE RELATED TO SWEETENER PREFERENCES IN 30 STRAINS OF MICEBachmanov A.A.¹, Li S.¹, Li X.¹, Lu K.¹, Tordoff M.G.¹, West D.B.², Ohmen J.D.², Reed D.R.¹, Beauchamp G.K.¹ ¹*Monell Chemical Senses Center, Philadelphia, PA;* ²*Pfizer Global Research and Development, Alameda, CA*

Positional cloning has determined that a taste receptor gene, *Tas1r3*, is identical to the mouse saccharin preference (*Sac*) locus. The goal of this study was to analyze the relationship between sequence variants of *Tas1r3* and sweetener preferences. We characterized saccharin preferences, and sequenced the DNA including and surrounding the *Tas1r3* gene of 30 inbred mouse strains. Sixty sequence variants were detected; several of them had significant associations with saccharin preference. In most of the strains, these variants formed two haplotypes, with all alleles being common either to high-preferring or low-preferring strains. In a few strains, the haplotypes were recombinant. Analysis of these recombinant haplotypes suggests that the best match between the sequence variants and saccharin preferences occurs at nt positions 135 (silent S45S) and 179 (missense I60T), with the I60T variant being most likely to affect the function of the T1R3 receptor. A substantial portion of the strain differences in saccharin preference remained unaccounted for by allelic variation at *Tas1r3*, and thus must be attributed to variation at other genetic loci. This indicates that sweetener preference has a complex genetic determination and emphasizes the importance of identification of these other genes involved in sweetener preferences, which is a goal of ongoing studies in our laboratory. Supported by NIH grants R01DC00882 (GKB), R03DC03509, R01DC04188 and R01DK55853 (DRR), R01AA11028 (MGT) and R03DC03853 (AAB).

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DROSOPHILA SWEET-TASTE GENE TRE IS IDENTICAL TO A GUSTATORY RECEPTOR GENE GR5A.

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Taste gene, Tre, that controls the gustatory sugar sensitivity in *Drosophila* was first discovered as a spontaneous mutation in wild population. P-element mutagenesis of Tre followed by molecular biological analyses of the mutants led to the identification of the gene as Gr5a, which belongs to a large seven-transmembrane receptor gene family specifically expressed in the gustatory or olfactory receptor neurons. A single amino acid substitution in the predicted second intracellular loop domain was found to be responsible for the spontaneous mutation in Tre. The results suggested that Tre encodes a sweet taste receptor in *Drosophila* and that the invertebrate and mammalian sweet taste receptors have evolved independently from different origins. Electrophysiological and behavioral analyses using Tre mutants or the transgenic flies were then carried out to characterize the sweet taste receptor.

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A GR RECEPTOR IS REQUIRED FOR RESPONSE TO THE SUGAR TREHALOSE IN TASTE NEURONS OF DROSOPHILA.

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Bioinformatics and genome analysis has led to the identification of the *Gr* genes, a large gene family that encodes candidate taste receptors in *Drosophila*. The *Gr* genes encode putative seven-transmembrane domain proteins, and many are expressed in the labellum, the primary external taste organ. We have found evidence that one *Gr* gene, *Gr5a*, encodes a taste receptor required for response to the sugar trehalose. Consistent with a role in trehalose detection, two different deletions that abolish expression of *Gr5a* result in a trehalose sensitivity defect as measured by single unit electrophysiology as well as a 2-choice behavioral assay. This defect is specific: physiological recordings from individual *Gr5a* mutant neurons show that they respond abnormally to trehalose but normally to another sugar, sucrose, suggesting that sucrose is received by another receptor on the same cell. Consistent with these results, the behavioral response of mutant animals is abnormal to trehalose but normal to sucrose. Both physiological and behavioral defects are rescued by supplying a wild type copy of *Gr5a* on a transgene. Rescue requires the presence of a wild type copy of *Gr5a* but not of the adjacent gene *Tre1*, previously reported to encode a trehalose receptor. Our findings support the idea that *Gr5a* encodes a taste receptor for trehalose. Currently, we are extending this work by examining the ligand-specificity of *Gr5a* and by performing a structure-function analysis of *Gr5a*.

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CALCIUM PUMPS, LIPID RAFTS AND GPI ANCHORED PROTEINS IN CHEMORESPONSE

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We have shown indirectly that the hyperpolarizing conductance of *Paramecium* in chemical stimuli is sustained by the plasma membrane calcium pump. We have cloned the genes for 4 isoforms of the pump, three of which (2,3,4) are expressed. (*Paramecium* genome project shows two partial sequences not discussed here.) Isoforms 2-4 are all 41-43% identical (amino acid level) with human isoform 4b, and 80-85% identical to each other at the DNA and amino acid levels. Like some mammalian pumps, the *Paramecium* pumps might associate with lipid rafts and/or cytoskeleton. Triton-X-100 extractions of the cell membrane results in separation of the calcium pumps primarily into the Triton insoluble phase. This is typical of proteins associated with either lipid rafts or cytoskeleton. Immunoprecipitation of the pumps results in 3 distinct immunoblot bands in the 110-135 kD range, two of which we can attribute to isoforms 2 and 3 through the use of HA and GFP tags in transformed cells expressing the fusion proteins, and to an antiserum specific for the N terminus of isoform 3. Confocal microscopy of control and transformed cells expressing tagged isoforms 2 or 3, show that isoforms 2,3, and 4 co-localize to the base of cilia. Finer analysis may reveal other locations. The GPI anchored folate chemoreceptor also localizes to the base of cilia, implying that the receptor and pump co-localize to lipid rafts. Supported by DC 00721, GM 59988 and VCCC.

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CHORDA TYMPANI (CT) NEURONS EXPRESS NEUROTROPHIN RECEPTOR GENES DIFFERENT FROM GREATER SUPERFICIAL PETROSAL (GSP) NEURONS

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The CT branch of the geniculate ganglion (GG) innervates taste buds on the anterior tongue, and the GSP branch innervates buds in the incisive papilla and the palate. Previous work suggested that neurons within the rat GG expressed different protein-tyrosine kinase receptors, referred to as *trks*. Moreover, the pattern of *trk* expression changed during pre- and post-natal development. In this study we examined the expression of *trkA*, *trkB*, truncated *trkB*, *trkC* and the non-specific receptor, *p75* in single GG neurons after labeling the CT nerve with a tracer (biotinylated dextran, BD) iontophoresed into fungiform papillae, or labeling the GSP nerve by injecting Fluoro-Gold into the incisive papilla. Frozen sections at 10 um were placed on slides and avidin-peroxidase was used to reveal the BD-labeled neurons, and immunohistochemistry to reveal GSP neurons. Labeled single neurons were dissected from the sections and subjected to RNA amplification followed by polymerase chain reaction with primers designed to reveal transcripts of the receptors. Of 48 neurons innervating fungiform papillae 44 contained the *trkA* transcript and no others. Of 24 GSP neurons only 2 contained the *trkA* transcript; 2 contained the *trkB* and 8 contained the *trkC* mRNA. None contained either the truncated *trkB* or *p75*. The data suggest variations in neurotrophin receptor expression in neurons innervating taste buds at different sites. Supported by NIH Grant DC 04837.

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THE ROLE OF cAMP AND Ca^{2+} IN THE EXCITATION AND ADAPTATION OF TASTE RESPONSES TO HCl

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The regulation of sour taste responses by cAMP and Ca^{2+} was studied by monitoring rat chorda tympani (CT) nerve responses to HCl and intracellular pH (pH_i) in polarized taste receptor cells (TRCs). One to 30 mM HCl stimulation increased CT responses and decreased TRC pH_i in a dose dependent manner. Lingual application of 8-chlorophenylthio (CPT)-cAMP (20 mM) enhanced the CT responses to HCl and the drop in TRC pH_i relative to control. The cAMP-induced sour taste excitation was amiloride-insensitive. Post-cAMP responses at zero current-clamp (0 CC) were enhanced at -60 mV and suppressed at +60 mV, indicating activation of an H^+ -conducting pathway. Lingual application of 150 mM ionomycin did not affect the initial amplitude of the CT response to HCl, but decreased it by 50% in 2 min, indicating rapid neural adaptation. In polarized TRCs, ionomycin alkalinized resting TRC pH_i reversibly and increased pH_i recovery from an NH_4Cl pulse by 400%. Post-ionomycin HCl stimulation caused a transient decrease in TRC pH_i that recovered spontaneously. The ionomycin-induced pH_i recovery is related to the presence of Na^+-H^+ exchanger isoform 1 (NHE1) in the basolateral membranes of TRCs and it is activated by an increase in intracellular Ca^{2+} . We conclude that (i) cAMP enhances the sour taste of strong acids by activating a Ca^{2+} - and amiloride-insensitive H^+ conductance and (ii) an increase in intracellular Ca^{2+} stimulates pH_i recovery that increases sensory adaptation to acids. Supported by NIDCD grant DC-00122.

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EXPRESSION OF EPITHELIAL SODIUM CHANNELS (ENACS) IN HUMAN FUNGIFORM PAPILLAE

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Epithelial sodium channels (ENaCs) are believed to mediate the perception of salty taste. The expression of the mRNA for the different ENaC subunits - alpha, beta, gamma and delta - in human taste buds was studied. Fungiform papillae were obtained surgically from three volunteers (ID code: A, B and C). Total RNA in papillae was extracted and reverse transcribed to yield cDNA, which was used as the template for PCR. Primers were designed so as to amplify the entire coding sequences of each of the four subunits. Two subjects (A and C) showed no detectable expression of alpha-ENaC, but they did express the beta, gamma and delta subunits. Subject B expressed all four subunits. Sequencing of the delta subunit of all three subjects showed that one subject (C) lacked a 120 bp segment. Sequencing of the alpha subunit of Subject B revealed a 57 bp deletion. This subject (B) demonstrated an inability to consistently and unambiguously identify sodium chloride and citric acid. Immunohistochemical studies, using commercially available antibodies to the four ENaC subunits, are in progress to determine their subcellular distribution within taste buds. The identification of delta ENaC subunits in gustatory tissue is a novel finding. Supported in part by a grant from the Department of Veterans Affairs.

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PACAP MODULATES POTASSIUM CURRENTS AND PROMOTES SURVIVAL OF OLFACTORY RECEPTOR NEURONS

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Neuroprotection is a promising strategy for the treatment of neurodegenerative diseases. Pituitary adenylate cyclase activating polypeptide (PACAP) is an important neuroprotective peptide, yet the underlying neuroprotective mechanisms remain to be elucidated. We grew acutely dissociated mouse olfactory receptor neurons (ORNs), as well as an immortalized olfactory placode derived cell line (OP6), in the presence and absence of 40 nM PACAP and measured the voltage-gated potassium currents (IK) by whole-cell patch clamp. Concurrently, an activated caspase fluorescent marker, CaspACE FITC-VAD-FMK, was used to label living cells undergoing apoptosis in a series of time points after dissociation. The images of fluorescence-positive cells were recorded by confocal microscopy and the survival rates were calculated. We found that (1) in untreated primary cultures, the IK density in ORNs was 48% larger than the IK density in cells grown in PACAP. In OP6 cells, the IK density was not significantly different in the presence or absence of PACAP. (2) In serum-free media, PACAP significantly reduced neuronal apoptosis in ORNs for short (<24 hours) but not extended (24 hours to 9 days) culture durations (unpaired Student's t test). Our results confirm previous work that PACAP is neuroprotective for ORNs¹ and suggest that the observed suppression of the increase in IK, may be a mechanism for PACAP's neuroprotective effects in ORNs. This work was funded by NIH NIDCD grant # DC02994 to MTL. 1. Hansel, et al. (2001) *J. Neurosci.* 21(13):4625

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MECHANISM UNDERLYING ODOR INHIBITION IN TOAD OLFACTORY RECEPTOR NEURONS.

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Odorants induce excitatory and inhibitory transduction currents in vertebrates, from fish to mammals. Excitation involves a cAMP-gated conductance and a Ca^{2+} -dependent Cl^- conductance (Cl^-_{Ca}), both generating a depolarizing receptor potential that increases firing. A Ca^{2+} -activated K^+ conductance (K^+_{Ca}) participates in inhibition, causing a hyperpolarizing receptor potential that decreases firing [Morales et al., 1994; Sanhueza et al., 2000]. The mechanism underlying odor-inhibition remained virtually unknown. We show here that the activation of the ciliary inhibitory K^+ conductance also depends on the cAMP cascade, by using diverse odor stimuli, pharmacological agents and caged second messengers. The activation of K^+_{Ca} was prevented by drugs affecting this cascade (LY83583, SQ22536). Using caged Ca^{2+} , we show that intracellular Ca^{2+} activates Cl^-_{Ca} (sensitive to μM DIDS and niflumic acid) as well as K^+_{Ca} (sensitive to nM IbTx and CTx). Both conductances reside in the cilia, since neither of them could be activated in ORNs devoid of cilia. Uncaged cAMP also activated both conductances, while uncaged $InsP_3$ had no effect. We also found that a somatic K^+ current can be activated by odorants (μM cadaverine or a mixture of pirazine, isovaleric acid and triethylamine) in a cAMP-independent manner. Our results suggest that odorants activate both, the excitatory Cl^-_{Ca} and the inhibitory K^+_{Ca} , by Ca^{2+} increases mediated by a cAMP cascade. Besides, a somatic K^+_{Ca} current may also contribute to the inhibitory response. Grants FONDECYT 2990003, 4000014 (RM), 1990938 and ICM P99-031-F (JB).

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THE SCENT OF DANGER: CHEMICAL ALARM SIGNALS AND ESCAPE FROM CANNIBALISM IN NEWTSSchar D.W.¹, Krug P.J.¹, Zimmer R.K.¹ ¹*Biology, UCLA, Los Angeles, CA*

Larvae of the California newt exhibit a chemically mediated anti-predator defense, demonstrating escape behavior and increased refuge use after detecting a cue from cannibalistic adults. Stream water was collected near free-ranging adults and induced alarm responses in 80 to 100% of the larvae. Solutions were prepared by bathing adults (in the field), and these stimuli also caused strong alarm reactions. Blockage of the adult cloaca with inert gel did not diminish bathwater bioactivity, indicating the alarm substance was not an excretory product. Swabs of adult backs, sides, and bellies were all highly active in solution, showing the chemical cue was released from adult skin. Reversed-phase HPLC analysis revealed a potent sodium channel blocker, tetrodotoxin (TTX), and related structural isomers present in skin swabs, and in bathwater at 10⁻⁷ M. A TTX standard was tested in the behavioral assay at concentrations from 10⁻⁷ to 10⁻⁹ M, along with equivalent dilutions of bathwater. At each concentration, bathwater and the corresponding TTX solution triggered an alarm response from the same number of larvae, with no subsequent sublethal toxicity. Two additional sodium channel blockers, saxitoxin (STX) and mu-conotoxin (CTX), were assayed, and neither substance caused an escape reaction. Larvae exhibited a minor sublethal response to CTX distinct from all other solutions, and had no reaction to STX. Taken together, our results show that newt larvae: (1) escape cannibalism by detecting toxins used in adult chemical defenses against snakes and other vertebrate predators, and (2) possess sensitive olfactory receptors for tetrodotoxin, mediating a complex series of behavioral reactions without any ill physiological effects.

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CHEMICAL ATTRACTANTS AS CHEMICAL DEFENSESJohnson P.M.¹, Paul V.J.², Cruz-Rivera E.², Derby C.D.¹ ¹*Biology, Georgia State University, Atlanta, GA;* ²*University of Guam, Marine Laboratory, Mangilao, GU*

Many organisms are known to produce chemical defenses that deter predatory behavior. However the mechanisms of these defenses - how they are interpreted by and function against the chemosensory systems of predators - are poorly understood. We are using sea hares (a well-studied group of gastropod molluscs that includes *Aplysia*) and their predators to address this issue. When attacked, sea hares release two defensive secretions from separate glands. The ink gland secretes a bright purple fluid and the opaline gland a highly viscous substance. In encounters between sea hares and portunid crabs from Guam, sea hare secretions provided a significant survival advantage but by an unusual mechanism. After secretions were released, the crabs stopped attacking the sea hares and appeared to be eating the secretions, suggesting that chemical defenses functioned by acting as feeding stimulants. We further tested this hypothesis using pure ink and opaline secretions of *Aplysia californica* against the sympatric spiny lobster *Panulirus interruptus*. Opaline and ink were behaviorally attractive to lobsters. Preliminary amino acid analysis showed that amino acids are at micromolar quantities in opaline and somewhat lower in ink. Since mixtures of amino acids are known to be potent feeding stimulants of crustaceans, this suggests the amino acids may at least in part account for the secretions' attractiveness. Preliminary electrophysiological recordings from antennular chemosensory neurons of lobsters support this idea by showing that neurons excited by amino acids are also highly excited by sea hare ink-opaline secretions. Supported by NSF (Pre-doctoral Fellowship and IBN-0077474)

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LARVAL REEF FISH COULD USE ODOR FOR DETECTION, RETENTION AND ORIENTATION TO REEFSAtema J.¹, Kingsford M.J.², Gerlach G.³ ¹*Boston University Marine Program, Boston University, Woods Hole, MA;* ²*School of Biological Sciences, University of Sydney, Sydney, NSW, Australia;* ³*Marine Resources Center, Marine Biological Laboratory, Woods Hole, MA*

While evidence is mounting that larval reef fish are active participants in the process of settlement, the sensory/behavioral mechanisms by which these cm-scaled animals return from their oceanic phase to the reefs remain unknown. On One Tree Island (Great Barrier Reef, Australia) we tested freshly trapped larvae in a large choice flume on the shore. Here, we present the first evidence that larval reef fish (primarily apogonids) that were approaching the time of settlement detect differences between ocean and lagoon water and use chemical signals to orient toward lagoon water. We demonstrate that they sniff actively with well-innervated noses. Attraction to lagoon water was not confounded by temperature differences. We also describe for ebb tide plumes of lagoon water extending many kilometres from reefs. Such plumes could provide chemosensory cues for settlement stages of reef fish that can swim efficiently and with great endurance. We argue that imprinting on reef odour could serve both retention near the natal reef and navigation toward reefs from greater distances. Supported by the University of Sydney and Boston University.

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ODOR PLUMES AND HYDRODYNAMICS: HOW CRAYFISH FIND ODOR SOURCESMoore P.A.¹ ¹*Biological Sciences, Bowling Green State University, Bowling Green, OH*

Many animals use chemical signals to acquire information about habitats. Each habitat has a unique hydrodynamic environment that is dependent upon the structure of that habitat. Differences in the hydrodynamics (i.e. turbulence) of an environment will be reflected in the fine-scale structure of chemical signals. The structure of this information is dependent upon specific features within a habitat, and the information in signals can be habitat specific. We quantified the spatial and temporal information in an aquatic odor plume in three different artificial stream habitats with different substrate types by measuring turbulent odor plumes with an electrochemical detection system and the orientation behavior of the crayfish, *Orconectes rusticus*. Our results imply that the information obtained from chemical signals may be limited in some habitats. These constraints on information may affect how organisms perform chemically mediated behaviors. A detailed analysis of orientation behavior supports the theory that crayfish orient differently to food sources in streams with different substrates. These results show that the hydrodynamics associated with chemical signal structure can greatly influence the temporal properties of orientation to food sources. This research was funded by grants from the NSF DAB, NSF Sensory Systems, and a BGSU TIE grant to PAM, and the University of Michigan Biological Station.

INTRAINDIVIDUAL CORRELATION OF CHEMOSENSORY EVENT RELATED POTENTIALS

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Chemosensory event potentials have become a widespread tool in examining olfactory disorders. Our aim was to examine the test-retest reliability in these potentials looking at a certain period of time. We examined 20 healthy volunteers with normal olfactory function at three test sessions; Mean time interval between session one and two was 6.8 days, 12.45 days between session two and three and 19.3 days between session one and three. Chemosensory event-related potentials were obtained the same way in every session. Olfactory stimuli were phenylethylalcohol and H₂S (4ppm), for chemosomatosensory stimulation CO₂ 60%vol/vol was used. Each stimulus was applied 15 times to each nostril and later averaged. Recording positions were Fz, C3, C4, Cz and Pz. The peaks P1, N1, P2 and P3 were analysed and based on this measurement base to peak Amplitudes A-P1, A-N1, A-P2, A-N2 and A-P3, peak to peak Amplitudes A-P1N1, A-N1P2, A-P2N2 and A-N2P3 and the latencies (in relation to stimulus onset) of T-P1, T-N1, T-P2, T-N2 and T-P3. Using Pearson's correlation coefficient we could demonstrate a high number of correlations, whereas latencies of the potentials showed highest correlations: e.g. phenylethylalcohol at Cz, first and third measurement, Latency P2: 0.526 left/0.427 right; H₂S: 0.479 left/0.509 right and CO₂ 0.578 left/0.638 right. Overall, looking at olfactory substances more correlations exist on the right side while CO₂ shows more correlations on the left side. We were able to demonstrate a high test-retest reliability in chemosensory event related potentials even though the reasons for differences in both nostrils are not fully explained and need further examination.

APOLIPOPROTEIN E E4-POSITIVE INDIVIDUALS SHOW INCREASED OLFACTORY EVENT-RELATED POTENTIAL LATENCIES AT 2-YEAR FOLLOW-UP

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The apolipoprotein E (apoE) E4 allele has been identified as a risk factor for Alzheimer's disease. Recent research has demonstrated that E4 positive individuals have deficits in the ability to identify and remember odors, as demonstrated by psychophysical measures of olfactory function. In addition, healthy E4 positive elderly persons demonstrate delayed latencies of the olfactory event-related potential (OERP) compared to those without the allele. The purpose of the present study was to investigate whether E4 positive individuals show accelerated decline in OERP latency compared to E4 negative persons. This decline would be demonstrated by an increase in OERP latency after 2-year follow-up testing. Olfactory and auditory ERPs were recorded from the Fz, Cz, and Pz electrode sites in E4 positive individuals and age and gender-matched E4 negative individuals in a single-stimulus paradigm, using amyl acetate as the olfactory stimulus and a 500 Hz tone as the auditory stimulus. Preliminary results suggest that E4 positive individuals demonstrate accelerated decline in the cognitive P3 component of the OERP, but less or no decline in earlier components. Supported by NIH grants DC02064 and AG04085 to CM. We thank Drs. Leon Thal, Robert Katzman, and Mark Bondi of the UCSD ADRC for genotyping, Charlie Morgan for expertise in olfactometry, and Rose Calhoun Haney for assistance.

A STUDY OF HYPOADDITIVITY USING DICHORHINAL STIMULATION

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Odor mixtures do not generally smell as strong as the sum of their individual components, a phenomenon known as hypoadditivity. Suppression of odor perception could occur at the level of the receptor or more centrally. For example the olfactory bulb is well set up to modify input from the receptor cells. We studied the possible involvement of the olfactory bulb by using a known counteracting pair of odors and delivering them to the same and different nostrils (dichorhinal stimulation). The psychological and physiological responses to pulses of the counteracting pair of odors - valeric acid and Veilex[®] 2 - were recorded using a psychometric test and EEG electrodes. Odor pulses (2% valeric acid and 0.01% Veilex[®] 2 in dipropylene glycol) were delivered using an air dilution olfactometer, in blocks of 15 x 100ms pulses (flow rate 3L/min). Subjects were required to indicate when they detected an odor pulse by pressing a button. The detection rate was expressed as a percentage of pulses delivered. The olfactory event-related potential (OERP) was recorded at Pz using EEG electrodes. When the two odors were delivered to the same nostril the N1-P2 amplitude of the OERP was reduced by 40.9 ± 2.8% (n=10). Delivering the odors to separate nostrils resulted in a suppression of 34.2 ± 3.2%. The perception of the odor was suppressed by 28.0 ± 6.4% and 20.2 ± 5.5% (n=10) when the two odors were delivered to the same and different nostrils respectively. There was no significant physiological or perceptual difference in the degree of odor suppression of the mixture when delivered to the same or different nostrils. Funded by Bush Boake Allen/International Flavors and Fragrances, New Jersey, USA

STEREOSPECIFICITY OF ELECTROPHYSIOLOGICAL AND SUBJECTIVE RESPONSES OF NICOTINE

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The electrophysiological (pattern reversal event-related potentials) and subjective (descriptive ballot) effects of smoking denicotinized cigarettes containing either R(1.99%), S(1.96%), or RS(2.08%) nicotine were studied in fifteen adult (age>21) volunteer smokers. Cigarettes containing only R nicotine could not be electrophysiologically or subjectively differentiated from denicotinized controls. Cigarettes containing RS nicotine produced subjective effects intermediate to those produced by S nicotine cigarettes and to those produced by control cigarettes. However, cigarettes containing RS nicotine produced electro-physiological effects comparable to those produced by cigarettes containing twice the amount of S nicotine. These data suggest that, although R nicotine itself is inactive, it synergistically interacts with S nicotine, enhancing the effects of the latter.

TRIGEMINAL EVENT-RELATED POTENTIALS: RELATION TO STIMULUS DURATION AND INTENSITY

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While widely used in studies on intranasal trigeminal sensitivity few data are available on potential effects of stimulus duration on the EEG-derived trigeminal event-related potential (ERP). The study aimed to develop a model describing relation between ERP components and both stimulus duration and stimulus intensity. Further, the study was designed to learn more about the characteristics of individual ERP components. Twenty healthy subjects participated (10 male, 10 female, 18-38 years). Trigeminal ERPs were recorded after stimulation with CO₂. Five CO₂ concentrations (45, 50, 55, 60, 65% v/v) and 5 stimulus durations (100, 150, 200, 250, 300 ms) were used. To reduce the duration of experimental sessions 9 of the 25 possible combinations were selected for each subject. ERP amplitudes and latencies of both, early (N1) and late components (P3) were measured. The relation between stimulus duration, intensity and amplitudes or latencies of trigeminal ERP components could be described using a power model. A linear relationship was found between stimulus intensity, amplitude N1, and amplitude P3, and between stimulus duration and amplitude P3. Further, a linear relationship was seen between intensity ratings, stimulus duration, and stimulus intensity. These data indicate that ERP components encode different stimulus characteristics. Specifically, later components of the trigeminal ERP not only encode stimulus intensity, but, other than earlier ERP components, also stimulus duration. Thus, later ERP components seem to reflect the integration of stimuli over relatively long periods which appears to be of high significance to the functioning of the intranasal chemosensory systems.

OLFACTORY PROCESSING AND MEDIAL FRONTAL CORTEX: ELECTROPHYSIOLOGICAL APPROACH

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Recent work from our laboratory has emphasized the importance of medial frontal cortex (mFCx) in olfactory learning. An electrophysiological approach was used to understand how the modifications of neuronal activity in this region might be related to learning of the odor-reward association. Neurons from piriform cortex (PCx) and mFCx were recorded simultaneously in anesthetized rats and responses to 6 odors evaluated. Whereas 39/90 PCx units responded to at least 1 odor, only 16/79 FCx units showed any response. When they did respond, neurons in mFCx were more selective. In a rapidly acquired behavioral task involving foraging for reward associated with an odor, odor responses were assessed before and after acquisition to evaluate learning-induced changes in mFCx. Only a few mFCx neurons showed initial olfactory responses; learning did not appear to affect these responses or induce new responses. Nevertheless, some neurons presented a sustained modification of their firing rate within the experimental context. Rats were trained on an odor-based Go/Nogo task requiring several training sessions. In this situation, some cells responded to the reward and/or to the odor. The results under anesthesia clearly indicate that there is a small population of neurons in mFCx that shows a spontaneous olfactory response. Whether these responses have learning-induced plasticity remains an open question. Our preliminary results from behaving animals suggest that they do, although additional experiments are necessary to understand the relative contribution of the experimental context and the CS.

NEURAL CORRELATES OF CORTICAL ODOR HABITUATION

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Piriform cortex neurons rapidly filter repetitive odor stimuli despite relatively maintained input from mitral/tufted cells. Previous work from our lab (Wilson, 1998) has shown that there is a short term synaptic depression (1-3 minutes) associated with this odor habituation. The purpose of the present study is to elucidate mechanisms underlying this form of non-associative neural plasticity using in vivo and in vitro preparations. Coronal slices (400 μ thickness, prepared and maintained using standard in vitro procedures) from young Long Evans rats were used for LOT-evoked field potential recordings from layer Ia. Field potentials were recorded before and after simulated odor stimulation of the LOT (80 ms 100 Hz trains repeated at 0.5 Hz with various total durations and differing current intensities). Results from this paradigm were compared with in vivo intracellular recordings from layer II/III neurons within piriform cortex of urethane-anesthetized rats stimulated with odorants. The time-course of short term depression of LOT synapses induced by both electrical and odorant stimulation methods was similar. In addition, the magnitude of the synaptic depression induced by the in vitro simulated odor stimulation of the LOT matched that of the depression evoked by the in vivo odor stimulation. This in vitro paradigm will now be used to dissect the mechanisms underlying in vivo cortical odor habituation. Funded by DC03906 to DAW.

OLFACTORY MEMORY AND THE MEDIAL FRONTAL CORTEX IN THE RAT

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Rats can learn to forage among three olfactory cues to associate one with reward in only 3 massed trials. The learning is achieved in less than 10 min and results in a memory trace lasting at least one week. To study the neuro-anatomical circuits involved in the memory formation we used immunoreactivity to the immediate early gene c-fos as a marker for neuronal activity induced by the associative learning. Selective increases in c-fos immunoreactivity occurred in the brains of trained rats (T) compared to yoked pseudo-trained (PT) controls in prelimbic and ventro-lateral orbital cortex as well as basal-lateral amygdala. Piriform cortex and other regions showed activation in both T and PT groups. The hippocampus was not activated under either condition. In subsequent experiments, injection of an NMDA receptor antagonist directly into the prelimbic cortex after learning induced amnesia 24h later, while similar injections into the hippocampus had no effect. These results suggest that prefrontal cortical regions are part of a neural circuit involved in the post-acquisition consolidation of memory for odor-reward association. (supported by CNRS UMR7102)

TASTE, TEXTURE, AND FAT REPRESENTATIONS IN THE PRIMATE ORBITOFRONTAL CORTEXVerhagen J.V.¹, Rolls E.T.¹, Kadohisa M.¹ ¹*Experimental Psychology, University of Oxford, Oxford, United Kingdom*

The primate orbitofrontal cortex contains the secondary and tertiary taste and olfactory cortices, and also receives somatosensory inputs (Rolls, 1997, 1999). In recordings in awake rhesus macaques we now report that some single neurons in the OFC have firing rates that depend on the viscosity of aqueous methyl cellulose in the range 1 - 10⁴ cPoise. Of these neurons, 56% were tuned to have parabolic response functions within this viscosity range, while 22% had increasing and 22% had decreasing response functions. Of these oral texture-sensitive neurons, 56% responded to taste stimuli. Of taste-responsive neurons, 29% also responded to viscosity. Thus neurons that show convergence between taste and texture are found in the orbitofrontal cortex, yet both are also represented independently. Such representations may be important in coding for all the sensory properties of a food in the mouth that influence its flavor (Rolls, 1999). A population of OFC neurons responds to fat in the mouth, and their responses are related to texture in that the same neurons can be activated by paraffin oil and silicone oil (Rolls et al, 1999). Of fat-sensitive neurons, only 63% responded to the methyl cellulose viscosity series. Thus fat can be detected and represented in the primate orbitofrontal cortex by a texture mechanism that is independent from the type of texture that is measured by viscosity, and which probably reflects the slickness of fat. Rolls, E.T. (1997) *Critical Reviews in Neurobiology* 11: 263-287. Rolls, E.T. et al (1999) *Journal of Neuroscience* 19: 1532-1540. Rolls, E.T. (1999) *The Brain and Emotion*. Oxford University Press: Oxford.

TEMPORAL ASPECTS OF GUSTATORY CODING OBTAINED FROM CORTICAL ENSEMBLES IN AWAKE RATSKatz D.B.¹, Simon S.A.¹, Nicolelis M.A.¹ ¹*Neurobiology, Duke University, Durham, NC*

In awake rats the content of gustatory cortical (GC) responses to tastants changes with post stimulus time. Here, we analyzed how perception may evolve across this time-course, using multivariate statistics on sliding windows of response time. Tastant responses were found to become increasingly discriminable across the first second following stimulus administration. This increase in discriminability was related to a rapid (within the first 0.5 to 0.7 sec) expansion of the multi-dimensional space required to describe the tastant responses. Although tastants could be discriminated using only the first burst of tastant-specific firing (which appeared approximately 200 msec after stimulus onset), the ease of discrimination greatly increased with time. Following this time of increasing discriminability, a regrouping occurred, wherein the similarity between aversive tastants (nicotine, quinine) began to rise. By 2.5 sec following tastant administration, GC responses to highly aversive tastants had become very similar to each other and distinct from those to highly palatable tastants. While some of this progression may be attributed to palatability-specific feedback from the somatosensory system, we suggest that the timecourse of GC responses starts earlier than orofacial responses, and thus reflects a process whereby the taste system first makes stimulus representations more distinctive, and then categorizes them on the basis of their reward value. Support: NIH DC-01065 & 00403 and DE-11121; Philip Morris Research Council.

DIFFERENT REACTION OF HUMAN BRAIN TO THE INVIGORATING AND RELAXING ODORSWang J.¹, Eslinger P.J.², Smith M.B.¹, Yang Q.X.¹, Ansari R.³ ¹*Radiology, Pennsylvania State University, Hershey, PA;* ²*Neurology, Pennsylvania State University, Hershey, PA;* ³*Technical Affairs, Quest International Fragrances Company, NJ*

The effects of smells on human behavior and performance are likely mediated by the emotional response and mood states that are stimulated by odors. To study how this process is executed in the brain, we focus this study on the brain responses to peppermint and lavender that generate mood known attributes (invigorating and relaxing). Nine normal adults (26±6 years old, 3 male and 6 female) received olfactory fMRI twice with each odor on a 3T MRI system. Statistical parametric maps of each group were created after one-sample t-test ($p < 0.001$). Two-sample and paired t-tests ($p < 0.01$) were processed to obtain the difference activation maps. Both odors elicited patterns of widespread brain activation involving frontal, temporal, insular, and parietal cortices. Primary olfactory cortices were activated. Subcortical activations were detected in the cerebellum, brain stem, basal ganglia, and thalamus. Statistical comparisons indicated that peppermint stimulation caused more widespread activation than lavender. The main difference is in the regions of insular, parietal and frontal cortices. In contrast, lavender elicited greater activation around piriform cortex. In conclusion, though brain activation patterns were similar in certain respects, lavender and peppermint elicited different profiles of neural reactivity, indicating that olfactory stimuli can have diverse and profound effects on emotion-processing, motor, sensory, and cognitive systems. (Supported by Quest International and Whitaker Foundation)

TEMPORAL LOBE OLFACTORY CORTEX ACTIVATION DURING SNIFFING AND VELOPHARYNGEAL CLOSURESabri M.¹, Kareken D.A.¹, Hector D.², Claus E.D.¹, Hutchins G.D.³ ¹*Neurology, Indiana University School of Medicine, Indianapolis, IN;* ²*Psychology, Indiana University, Bloomington, IN;* ³*Radiology, Indiana University School of Medicine, Indianapolis, IN*

Imaging suggests that sniffing alone evokes piriform activation. However, no work has directly compared olfactory stimulation during sniffing (S) and velopharyngeal closure (VPC), which does not require sniffing. We studied odor and control stimulation during S and VPC with PET in 5 young, normosmic subjects. Seven familiar odors (or blank controls) were delivered in 2 sec bursts through an olfactometer attached to a birhinal nasal cannula. Image analysis used SPM99 and random effects analysis ($p < .05$, uncorrected), and focused on a 16mm diameter sphere just anterior to piriform cortex-- an area that we found to activate in prior work with other subjects. Sniffing odors compared to control sniffing again activated this region ($p < .004$). VPC with odors compared to control VPC activated the same area ($p < .001$), but VPC produced a slightly stronger effect ($p < .02$). By contrast, control sniffing produced greater activation than control VPC ($p < .01$) in the piriform area, orbital cortex, and anterior/inferior insula. Thus, control sniffing activated more olfactory areas than just piriform cortex. Breathing technique may therefore modulate olfactory activation in PET, perhaps because sniffing actively inspires environmental air, which cannot always be precisely controlled. Some PET studies may fail to detect piriform or proximate activation for this reason. *Supported by AG16889; Thanks to Stephen Warrenburg and IFF for providing odors.*

REDUCED TASTE INTENSITY PERCEPTION IN PATIENTS WITH IPSILATERAL OR BILATERAL INSULAR ATROPHY DUE TO PRIMARY PROGRESSIVE APHASIA

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Primary Progressive Aphasia (PPA) is a focal dementia characterized by gradual dissolution of language function, which may be of a non-fluent, fluent or mixed typology (Mesulam, 2001). Non-fluent PPA is associated with brain atrophy in the dominant frontal operculum and insular cortex, including the primary taste region. Atrophy is generally restricted to the dominant hemisphere in early stages but becomes bilateral as the disease progresses. To investigate the relationship of taste intensity perception with insular brain atrophy we compared taste and greyness intensity estimation in patients with PPA and age-matched control subjects. Voxel-based morphometry was performed on patient T1 MRI brain volumes to measure brain atrophy. A repeated measures analysis of variance showed that when taste was applied separately to the left or right side of the tongue, the perceived intensity reported by PPA subjects was significantly less than that reported by subjects in the control group for both sides of the tongue. No group difference was found for whole mouth stimulation. The control group, but not the PPA group, rated the stimulus applied to the right side of the tongue as significantly more intense. However, preliminary analysis of anatomical data suggests that 1) when atrophy is restricted to the left hemisphere, taste intensity perception is reduced on the ipsilateral side of the tongue and 2) there is an overall negative relationship between degree of insular atrophy and taste intensity perception. These results are consistent with Pritchard et al., 1999 in suggesting ipsilateral organization of the human gustatory system. Funded by NIA PHS AG13854 awarded to D. Small.

OLFACTORY MARKER PROTEIN IS EXPRESSED IN THE VISUAL CORTEX OF RATS AND CATS

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Olfactory marker protein (OMP) is a protein characteristically found in mature olfactory sensory neurons. We report here the ectopic expression of OMP in the visual cortex of postnatal rats and adult cats using RT-PCR and nested PCR followed by sequencing. Semiquantitative estimations reveal that the OMP mRNA expression level in the visual cortex is lower (approx. 0.1%) than in olfactory epithelium. The expression level does not change postnatally in the visual cortex of controls; however after visual cortex laser lesion the expression is lower in the lesion site, where fewer neurons are present. We found OMP mRNA is expressed in all investigated neuronal tissues (cerebellum, olfactory bulb, lateral geniculate nucleus) at low level but also at even lower abundance in non-neuronal tissues like kidney, liver, testis, intestine and muscle, however all of those tissues are innervated. From our data we cannot say whether it is expressed in neurons or in glia or endothelial or other cells. However, the fact that OMP mRNA is also expressed in the neuron derived cell line Odora, but not in the non-neuronal cell culture Hek293 supports the argument that OMP seems to be a general neuronal marker. The unchanged expression level in the visual cortex between pre-eye and post-eye opening indicates it to be activity-independent. We discuss a function of OMP in relation to neuronal turnover and survival. Financially supported by Forum F208/00 M122/13 (2000) and SFB509 TP C4. We thank Prof. Dr. Albert I. Farbman for helpful discussions and review.

CEPHALIC PHASE SALIVARY RESPONSE DIFFERENCES CHARACTERIZE LEVEL OF FOOD NEOPHOBIA

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Cephalic phase responses are rapid physiological processes, stimulated by the sensory properties of foods, that prime the body to better absorb and utilize ingested nutrients. The pre-ingestive flow of saliva following sensory stimulation provides a quantitative index of responsiveness to foods. Past research addressing differences between food neophobics (those individuals reluctant to try new foods) and food neophiles (those individuals particularly willing to try new foods) suggests difference may exist in cephalic phase salivary responses between these groups. The saliva flow of 90 participants was measured for a 3-minute period following exposure to either a lemon, funistrada (a fictitious food), or a non-food control stimulus. Participants were divided into 3 groups (data tri-split) based on level of food neophobia, as measured by the Food Neophobia Scale (FNS). Regardless of the stimulus condition, neophobics showed decreased salivation, and neophiles showed increased salivation, in relation to the average group. Only the average group showed salivation differences based on stimulus type, with low salivation in the control condition, moderate salivation in the funistrada condition, and high salivation in the lemon condition. These results indicate an associated physiological mechanism associated with food neophobic tendencies.

GENERALIZABILITY VS. SPECIFICITY OF PSYCHOPHYSICAL RATINGS MADE BY FOOD NEOPHOBICS AND NEOPHILES ACROSS ALL SENSORY DIMENSIONS

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Individual differences in human food neophobia influence the evaluation of tastes and odors. Frank and van der Klaauw (1995) reported that food neophobics (those individuals reluctant to try novel foods) rated tastes and odors as less pleasant and more intense than food neophiles (those individuals particularly willing to try novel foods). Also, when asked for a "just right" intensity for a number of tastants, food neophobics judged the tastants to be "just right" at a much lower perceived intensity than the food neophiles. The present study was designed to assess whether psychophysical ratings by these two groups are noted only for taste and odor stimuli, or are more general in scope. Participants (n=111) rated taste intensity (applesauce, vanilla pudding, funistrada), odor intensity (coffee, orange, vanilla), visual brightness (100L, 200L, 300L), auditory intensity (25db, 40db, and 60db for a 4000Hz tone), and tactile hardness (cotton, wood, sponge) on a 21 point scale specific to each sensory modality. In addition, participants completed the Food Neophobia Scale, and were placed into one of three data analysis groups (data tri-split) based on their scores. Confirming past research, food neophobics rated taste and odor stimuli as significantly more intense than neophiles. However, the results showed no significant difference between the groups in their ratings of light intensity, auditory intensity, or tactile hardness. Results indicate neophobia-related alteration of psychophysical ratings specifically in relation to taste and odor stimuli, providing no support for the contention of a more general neophobic personality trait or general sensory ratings bias.

REFRESHMENT IS DISTINGUISHED BY A DISCRETE SUBJECTIVE CHANGE

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We hypothesized that refreshing beverages are distinguished by their ability to induce a subjective experience of discrete positive change. In Exp. 1, 127 participants consumed 8 oz. of Lipton Iced Tea or Starbucks Iced Coffee at either 70 F or 40 F. They then responded to a yes/no "do you feel different" (FD) question, and rated their refreshment and liking on 9point scales. Rated refreshment was greater for tea (5.2) than coffee (3.9), $F(1,123)=8.3, p<.05$ and greater for cold (5.1) than warm (4.0) beverages, $F(1,123)=6.6, p<.05$. Similarly, FD judgments for positive responses (i.e. w/liking ratings > 4) were provided more often for tea (66%) than coffee (40%), $\chi^2=5.8, p<.05$ and more often for warm (45%) than cold beverages (64%) $\chi^2=3.0, p<.05$. Exp. 2 investigated the importance of the discrete wording and timing of the FD question. 418 participants consumed 8 oz. of Lipton Iced tea, Coca-Cola, Spring Water, or Starbuck's Iced Coffee. Refreshment ratings were higher for Iced Tea (6.8) and Water (7.2) than for Iced Coffee (5.8) and Coca-cola (5.9), $F(1,417)=10.51, p<.05$. When given right after consumption, a discrete yes/no FD question elicited more positive responses for refreshing beverages (Water- 60% and Tea- 71%) than less refreshing beverages (Iced Coffee-44% and Coca-Cola- 40%), $\chi^2=4.8, p<.05$. Strikingly, a continuous FD measure revealed no such effect, $F(1,173)=1.6$, and neither measure revealed differences when given after a brief delay, $\chi^2=1.5$ ~, $F<1$ respectively. These findings suggest that refreshing beverages elicit a discrete experience of feeling different that can be captured by a yes/no "do you feel different" question given immediately after beverage consumption. This work was funded by Unilever.

MODULATION OF THE HUMAN ACOUSTIC STARTLE REFLEX BY TEA AROMAS: A COMPARISON OF ASIAN AND NON-ASIAN SUBJECTS

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Vrana et al. (1988) have established that the magnitude of the eyeblink response to a startle stimulus is an objective measure of affective states in humans. Subsequent work (e.g., Kaviani et al., 1998) has shown that hedonically valent olfactory stimuli are an effective means of modulating the startle reflex. In the present study, black and green tea aromas, as well as pleasant and unpleasant aromas, were presented to a group of 20 Asian (green tea experienced) and 20 non-Asian (green tea naive) subjects. Both groups showed a similar modulation of the startle reflex for the pleasant and unpleasant aromas in a direction consistent with previous work: pleasant aromas inhibited the startle response, while unpleasant aromas potentiated it. Non-Asian subjects had a statistically significant inhibition of the startle reflex for warm black tea aromas ($p<.05$), and a neutral response to cold black tea aroma and both hot and cold green tea aromas. Asian subjects had a statistically significant inhibition of the startle reflex when exposed to both hot green and black tea aromas ($p<.01$), and a neutral response to the cold tea aromas. These results support the hypothesis that the affective influence of aromas is learned, as the Asian subjects were resident in the UK and consumers of both black and green teas, while the non-Asian subjects were consumers of black and not green tea. This work was funded by Unilever.

EFFECTS OF AMBIENT FLORAL ODORS ON FAMILY ACTIVITIES AND SELF PERCEPTIONS

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The objective of this study was to determine the long-term anxiolytic effects of a floral odorant in a natural environment. For one week a floral odorant was placed in the households of one hundred families. For two nonconsecutive weeks, on a daily basis, each family member rated their degree of relaxation on an analog scale. In a randomized fashion, families were presented with the odorant in either the first or second week with the nonodorant week acting as a control in each family. The floral odorant enhanced relaxation and reduced stress for each family member by 5% compared to the no-odor control week ($p=.03$) with a greater effect, the larger the size of the family (18.5% for a family of 5). In a natural environment, hedonically positive, floral odorant can induce a sustained anxiolytic effect.

AMELIORATING AGRICULTURAL ODORS: DOWN ON THE FARM

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Agricultural odors are much more than a nuisance. Indeed, swine odors from four farms resulted in a financial judgment by a US court of \$100,000 each to 52 neighbors who were unable to use their property, in part, because of the malodors. Stimulated by these issues and basic research questions concerning interactions of odorants, we evaluated methods to reduce or change malodors associated with swine farming. These included sensory cross-adaptation, e.g., presenting pleasant-smelling esters with swine slurry, manipulations of swine slurry itself, e.g., adding powdered activated charcoal (PAC) and placing additives in the feed, viz., copper chlorophyllin complex (CCC). Initially, work was conducted in the laboratory; however, our most recent phase required a more life-like situation. To this end we trained a panel to evaluate odors associated with swine production and conducted sensory evaluations on a small (~ 110 head) swine production facility. Fourteen evaluations were performed under various experimental conditions, e.g., with or without PAC, with or without CCC, etc. Results indicated that adding PAC to swine slurry had a positive impact on overall intensity and unpleasantness of the odors, especially downwind from the animals. Although CCC may have been effective under laboratory conditions, results from on-farm tests revealed that CCC had minimal impact on overall odor. Analytical tests of odor samples revealed significant reductions in myriad malodors. These results suggest alternatives to traditional swine farming that may reduce overall odor production, especially as perceived by neighbors. Supported by a grant from the Pennsylvania Department of Agriculture.

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Umami Taste

BEHAVIORAL EVIDENCE FOR A ROLE OF GUSTDUCIN IN UMAMI TASTE

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The taste perception of glutamate (MSG) has been termed "umami". A characteristic feature of umami taste is its potentiation by 5'-ribonucleotides. A truncated form of mGluR4 (taste-mGluR4) has been identified in taste cells and implicated as an umami taste receptor. When expressed in heterologous cells, taste-mGluR4 decreases intracellular cAMP, however, the G protein involved has not been identified. -Gustducin is a G protein subunit that activates phosphodiesterase (PDE) to decrease intracellular cAMP in taste cells. Gustducin is known to mediate bitter taste, but its role in umami has not been examined. In this study we used standard 2-bottle preference tests on gustducin knockout (KO) and wild type (WT) mice to compare taste preferences for ascending concentrations of MSG and MSG plus 1 mM IMP. Statistical comparisons between KO and WT mice revealed that WT mice strongly preferred 30 and 100 mM MSG to water ($p < .01$), however, KO mice showed no preference for any concentration of these compounds. Similar results were obtained for MSG plus IMP. Denatonium was used as a control, and as expected, WT mice avoided denatonium significantly more than the KO mice. These data suggest that gustducin may play a role in umami taste transduction. Supported by NIH grants DC03013 to SCK and DC03055 to RFM.

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Umami Taste

DISCRIMINATION BETWEEN TASTES OF MONOSODIUM GLUTAMATE AND GLUTAMATE AGONISTS IN RATS.

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The taste of monosodium glutamate (MSG) has been called "umami". A truncated form of mGluR4 (taste-mGluR4) has been identified in taste cells and has implicated as an umami taste receptor by conditioned taste aversion (CTA) studies. An NMDA-like ionotropic receptor has also been proposed for "umami" taste but CTA studies have been less supportive of this idea. In a CTA paradigm rats are conditioned to avoid ingesting substances that have similar tastes. We used a taste discrimination procedure to determine if Sprague-Dawley rats could detect differences between the tastes of MSG and either NMDA, L-AP4 (an mGluR4 agonist) or aspartic acid (ASP), another amino acid with a strong umami taste. Amiloride (30 μ M) was added to all solutions to reduce the taste of sodium ion. Detection thresholds of MSG, NMDA, and ASP were all between 1-5 mM whereas L-AP4 could be detected as low as 0.001-0.005 mM. Rats could readily discriminate between the tastes of NMDA (10-100 mM) and MSG (10-100 mM) at all concentrations but they had difficulty discriminating between MSG (10-100 mM) and ASP (10-100 mM). Rats could readily discriminate between MSG (10-100 mM) and low concentrations of L-AP4 (0.01-1.0 mM) but were less proficient when the concentrations of L-AP4 were greater than 2.5 mM. These data indicate that mGluR4 receptors may be involved in the perception of umami taste but that other taste receptors may also be activated by MSG. Supported by NIH grant DC03013 to SDR.

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Umami Taste

GENERALIZATION OF CTA BETWEEN MONOSODIUM GLUTAMATE AND SWEET SUBSTANCES IN RATS.

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Monosodium glutamate (MSG) has a unique taste called "umami" which is potentiated in the presence of 5' monophosphates. Umami taste is thought to be detected by a truncated form of mGluR4 (taste-mGluR4) on the apical membrane of taste receptor cells. However, conditioned taste aversion (CTA) studies have consistently found that the taste of MSG generalizes to sucrose when amiloride is added to the solutions. Amiloride is a sodium channel blocker that reduces the intensity of the sodium component of MSG. Moreover, nerve recording studies have shown that the potentiation between MSG and IMP is reduced by *Gymnema sylvestre*, a sweet antagonist. Sako and Yamamoto (1999) have suggested that MSG may activate sweet receptors on taste receptor cells. We used CTA to determine if the taste of MSG generalizes to other sweet substances including the natural sugars sucrose and glucose, and the artificial sweeteners saccharin and SC 45647. All solutions contained 30 μ M amiloride. CTA to each sweet substance generalized to MSG and a CTA to MSG generalized to each of the sweet substances. These data support the hypothesis that MSG, in addition to activating glutamate receptors, may also activate sweet receptors in the apical membrane of taste receptor cells. Supported by NSF grant 9982913 to ERD.

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Umami Taste

DOUBLE-LABELING C-FOS MRNA AND PROTEIN IN THE RAT SOLITARY NUCLEUS AFTER SUCROSE AND MSG INGESTION.

Gropp J.K.¹, Stapleton J.R.¹, Barnes C.L.², Delay E.R.¹ ¹*Neuroscience Program, Regis University, Denver, CO;* ²*Physical Therapy, Regis University, Denver, CO*

Previous studies have indicated an overlap in the chemotopic maps of c-fos expression generated in response to multiple tastants (Travers et al., 1996), and more specifically to monosodium glutamate (MSG) and sucrose (Stapleton et al, 2000; Gropp et al, 2001). In the present study, the technique of double-labeling c-fos mRNA and c-fos protein is being used to study whether individual neurons in the solitary nucleus of the rat respond to multiple tastants. Double labeling of c-fos like immunoreactivity (c-FLI) is possible because of the differential time courses of expression of mRNA and protein. Rats are entrained to drink in two separate periods, separated by one hour. On test day, one stimulus (150mM sucrose with 50 μ M amiloride) is presented, followed by a second stimulus (150mM MSG with 50 μ M amiloride). The rat is perfused transcardially 15 min. later and the brain is removed. C-fos mRNA is labeled by fluorescent in situ hybridization (ISH) and c-fos protein by fluorescent immunocytochemistry (ICC). Sections (30mm) are processed with standard ISH followed by ICC techniques using ABC signal amplification and TSA development. c-FLI neurons were identified in the caudal gustatory region of solitary nucleus following stimulation with sucrose and MSG, an area previously reported to have cells that respond both to MSG and sucrose. Double-labeled cells responding to both stimuli were identified. Single-labeled cells responding to one or the other stimulus were also identified. Supported by NSF grant 9982913 (ERD) and NIH/NIDCD, P30 DC04657 (Diego Restrepo)

HUMAN RECEPTORS FOR SWEET AND UMAMI TASTE

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The three members of the T1R class of taste-specific G-protein-coupled receptors have been hypothesized to function in combination as heterodimeric sweet taste receptors. Here we show that human T1R2/T1R3 recognizes diverse natural and synthetic sweeteners. In contrast, human T1R1/T1R3 responds to the umami taste stimulus glutamate, and this response is enhanced by 5'-ribonucleotides -- a hallmark of umami taste. The ligand specificities of rat T1R2/T1R3 and T1R1/T1R3 correspond to those of their human counterparts. These findings implicate the T1Rs in umami taste and suggest that sweet and umami taste receptors share a common subunit.

NITRIC-OXIDE AS A POSSIBLE GAIN CONTROL IN THE OLFACTORY SYSTEM

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The presence of nitric oxide (NO) in the vertebrate olfactory bulb and invertebrate antennal lobe (AL) is well documented. However, its functional role remains largely a mystery. NO readily diffuses through cell membranes in a non-selective manner and is released in response to odor presentation. Its potential targets may be distributed and embedded in several processing pathways. One potential use for a globally reaching agent such as NO may be gain control. In this case, NO release would decrease the sensitivity of the olfactory system, making odor cues less salient but not necessarily less discriminable. Alternatively NO could act as a short-term modulator of perceptual acuity affecting odor discriminability. We investigate these hypotheses by manipulation of NO level in the AL of the adult sphinx moth *Manduca sexta*. Adult moths can be conditioned to respond to an odor with a feeding response. Subsequent testing with an array of novel odors allows assay of odor discriminability. We couple assay of conditioned response to odors with injection of pharmacological agents in both ALs before conditioning and/or testing in a 2x2 factorial design. Here we show that NO blockade increases responsiveness to the conditioning odor and test odors with little effect on odor discriminability. This increased responsiveness can be corrected by simultaneous injection of an NO donor (SNaP). In conclusion, consistent with the NO gain control hypothesis, NO appears to affect odor salience but not odor discriminability. This work was supported by NIH-NCRR (9 R01 RR14166-06) to BHS & (1 R03 DC05535-01) to KCD.

NO ACTS AS A COMPLEX MODULATOR OF RAT OLFACTORY RECEPTOR NEURON ACTIVITY

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Nitric oxide (NO) is a neuronal messenger in the vertebrate olfactory epithelium. There is controversy about its effects on the cyclic nucleotide-gated (CNG) transduction channels which localize principally to the cilia, but are also found in the dendrite, soma and axonal growth cones of the olfactory receptor neurons (ORNs). NO has been reported to activate olfactory CNG channels of the salamander, but it inhibited the CNG conductance of rat ORNs. To further investigate this issue, we recorded the electrical responses of rat ORNs to NO-stimulation using two complementary techniques: Extracellular recordings from the isolated olfactory epithelium and whole-cell patch clamping of dissociated ORNs. Our principal findings are: 1. NO exerted a prominent inhibitory effect on the spontaneous ORN activity in extracellular recordings. The spike inhibition was dose-dependent, reversible and started 10-15 s after stimulus onset. 2. During the initial delay, a transient increase in the spike rate was frequently observed, suggesting that NO may cause both excitation and inhibition, with inhibition being the stronger and longer-lasting effect. 3. High concentrations of NO (10 mM sodium nitroprusside in the patch pipette) activated the CNG conductance. 4. If the CNG conductance was already activated by cAMP dialysed through the patch pipette, a subsequent stimulation with a short pulse of NO generally enhanced the associated current, but did not inhibit it. As NO may also activate an inhibitory calcium-dependent potassium conductance and raise cGMP levels, it appears to affect ORN spike rates through several pathways and could play a role as a complex modulator of ORN activity *in vivo*. Grants Fondecyt 1990938 and ICM P99-031-F (JB).

SYSTEMIC L-NAME ATTENUATED LITHIUM-INDUCED C-FOS EXPRESSION IN THE BRAIN, BUT NO EFFECT ON THE ACQUISITION OF LITHIUM-INDUCED CONDITIONED TASTE AVERSION IN RATS.

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Intraperitoneal lithium chloride induces conditioned taste aversion (CTA), partly through activation of HPA axis, and c-Fos expression in the brain regions implicated in CTA learning, such as the hypothalamic PVN, nucleus tractus solitarius (NTS), central amygdala (CeA). It was reported that manipulation of brain nitric oxide level modulates lithium-induced CTA learning. To investigate if this behavioral modulation by nitric oxide were accompanied by modulations of brain c-Fos expression and activation of HPA axis, we performed c-Fos immunohistochemistry in the brain, CTA test, and plasma corticosterone assay with N(G)-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, pretreatment followed by lithium chloride. In the results, intraperitoneal lithium chloride dose-dependently (3ml/kg, 6ml/kg, 12ml/kg, 0.15M) induced c-Fos expression in the PVN, NTS and CeA. L-NAME (30mg/kg) given 30 min prior to each dose of lithium chloride significantly decreased c-Fos-ir cells in all three brain regions, however, L-NAME did not affect CTA learning by high dose lithium (12ml/kg) and rather potentiated CTA by low dose lithium (3ml/kg). Plasma corticosterone remarkably increased by lithium chloride and L-NAME pretreatment did not block this increase at all. These results suggest that nitric oxide may be involved, at least in part, in the neuronal activation of the brain regions, but not in the activation of HPA axis, during the acquisition of lithium-induced CTA, and the strength of CTA acquisition is not solely correlated with the numbers of c-Fos expressing cells in each brain region. Supported by KISTEP(JWJ).

381 Poster Neurotransmitters and Neuromodulators**EXPLORING NEURONAL AND MOLECULAR MECHANISMS THAT MEDIATE ODORANT-STIMULATED NITRIC OXIDE PRODUCTION IN THE ANTENNAL LOBE OF MANDUCA SEXTA**Wilson C.¹, Collmann C.¹, Nighorn A.¹ ¹ARLDN, University of Arizona, Tucson, AZ

The unique anatomy of olfactory glomeruli and the expression of nitric oxide (NO) synthase and NO-sensitive proteins in the glomerular neuropil of many species suggests that NO signaling participates in olfactory information processing. Using immunocytochemistry in the moth *Manduca sexta*, we show that NO synthase is expressed in the axons of olfactory receptor neurons, and that soluble guanylyl cyclase is expressed in a subset of antennal lobe neurons including projection neurons, GABAergic local interneurons, and the single serotonergic neuron. Using the NO marker, diaminofluorescein diacetate (DAF-2DA), we show that stimulating the antenna with odorants causes NO production in all glomeruli. One model to explain this phenomenon is that odorant-stimulated activity in a single glomerulus excites multiglomerular neurons including either GABAergic local interneurons or the serotonergic neuron, resulting in NO synthase activation in unstimulated olfactory receptor neurons. To begin to test this hypothesis, we used degenerate oligonucleotide RT-PCR to identify fragments of three ionotropic GABAA receptors, one metabotropic GABAB receptor, and two serotonin receptors. The expression of these receptors on olfactory receptor neurons and their involvement in activating NO synthase will be examined. Supported by National Institutes of Health grant DC04292

382 Poster Neurotransmitters and Neuromodulators**EXPRESSION AND FUNCTION OF NPY IN THE RAT OLFACTORY BULB**Blakemore L.J.¹, Alshingiti A.M.¹, Levenson C.W.¹, Trombley P.Q.¹ ¹Program in Neuroscience, Florida State University, Tallahassee, FL

Several neurologic and neuropsychiatric disorders have been linked to the olfactory bulb (OB) and neuropeptide Y (NPY). Although NPY is the most abundant neuropeptide in the brain, with its highest levels in the OB, little work has addressed the importance of NPY to OB function. In this study, we used, molecular, immunological, and electrophysiological techniques to explore NPY expression and function in the OB. Northern analysis revealed that NPY mRNA is expressed at birth and that levels increase dramatically during development; parallel results were seen in culture. Radioimmunological and immunocytochemical analyses revealed significant NPY protein expression in cultured neurons. Electrophysiology demonstrated that NPY can dramatically suppress excitatory transmission in a subset of neurons. Voltage- and current-clamp analyses suggest that the effects of NPY are not postsynaptic. NPY did not directly affect glutamate-evoked currents, input resistance, or the ability of postsynaptic neurons to fire single or repetitive action potentials. Although the effects of NPY on synaptic transmission would appear to be presynaptic, initial results from experiments on calcium or potassium channels are equivocal and require further analysis. Collectively, these results demonstrate a developmental pattern of robust NPY expression. NPY-mediated modulation of excitatory synaptic circuits in the OB may contribute to odor information processing. Disruption of this type of modulation may contribute to neurologic and/or neuropsychiatric disorders involving NPY and the OB. Supported by NIH and FSU.

383 Poster Neurotransmitters and Neuromodulators**ACTIVATION OF PURINERGIC RECEPTOR SUBTYPES DIFFERENTIALLY MODULATES MOUSE ORN ODOR RESPONSIVENESS**Hegg C.C.¹, Lucero M.T.¹ ¹Physiology, University of Utah, Salt Lake City, UT

Extracellular ATP, an important signaling molecule in numerous systems, is released by neurotransmitters, cell damage, and potentially, by noxious odors. Released ATP binds purinergic receptors and stimulates an increase in intracellular calcium. Our previous studies showed that ATP differentially modulates odor responsiveness in ORNs with suppression occurring more frequently than enhancement. Here we determined that odor response suppression or enhancement depends on activation of specific purinergic receptor subtypes. We found that (1) P2Y receptor selective agonists (UTP and ADP- S) caused suppression of the odor response, where the response of an ORN to co-application is less than the sum of the responses to the individual components, and (2) a P2X receptor selective agonist (-MeATP) caused enhancement, in which the response to co-application is larger than the sum of the individual components. A few studies have provided indirect evidence that neurotransmitters modulate odor sensitivity at the level of the ORN. This is the first report with direct evidence that a neuromodulator, ATP, can differentially modulate the odor responsiveness of ORNs. The complement of P2X and/or P2Y receptor subtypes expressed in the ORN will determine whether the odor response is enhanced or inhibited in the presence of ATP. The predominantly suppressive effect of ATP on odor responses could play a role in the reduced odor sensitivity that occurs during acute exposure to noxious fumes and may be a novel neuroprotective mechanism. This research was supported by NIH NIDCD DC04953 and DC02994.

384 Poster Neurotransmitters and Neuromodulators**DOPAMINE INHIBITS ODOR RESPONSIVENESS AND EXCITABILITY IN MOUSE ORNS**Hegg C.C.¹, Lucero M.T.¹ ¹Physiology, University of Utah, Salt Lake City, UT

Few studies have investigated modulatory effects of neurotransmitter agonists acting at distinct neurotransmitter receptors on olfactory receptor neurons. Previously, we showed that dopamine (DA) inhibits the hyperpolarization-activated current (I_h) via D2 dopamine receptors (1). Here, we used a mouse olfactory epithelium slice preparation and calcium imaging to investigate the effects of dopamine on odor responses. DA decreased the amplitude of both odor and high K⁺-evoked calcium transients, effects that were reversed with the D2 receptor antagonist sulpiride. This suggests that DA decreased the odor sensitivity and excitability of ORNs. Reduced excitability was further confirmed by the observation that DA decreased the probability of obtaining a high K⁺ response. DA could reduce odor responsiveness by activating Gi-coupled D2 DA receptors, resulting in inhibition of adenylyl cyclase activity. Reduced cAMP production would inhibit I_h and hyperpolarize the cell. Hyperpolarization could reduce odor responses but should not reduce high K⁺ responses. Thus we were surprised to see DA-evoked decreases in high K⁺ responses. However, a recent finding that D1 agonists reduce the amplitude of sodium currents in ORNs (2) suggests that dopaminergic modulation of ORN excitability and odor sensitivity is complex. Our findings provide evidence that dopamine reduces the sensitivity and output of ORNs to odorants. Funded by NIH NIDCD grant# DC02994 to MTL. 1. Vargas & Lucero, (1999) *J. Neurophys.* 81:149-158. 2. Wetzel et al., (2001) Phosphorylation of voltage-gated ion channels in rat olfactory receptor neurons. *Eur. J. Neurosci.* 14(7):1056-1064.

ELECTROPHYSIOLOGICAL, IMMUNOCYTOCHEMICAL, AND MOLECULAR ANALYSES OF KAINATE RECEPTORS IN THE RAT OLFACTORY BULB

Davila N.G.¹, Horning M.S.¹, Blakemore L.J.¹, Houpt T.A.¹, Trombley P.Q.¹ ¹*Biological Science, Florida State University, Tallahassee, FL*

Although glutamate mediates transmission at most, if not all, excitatory synaptic circuits in the rat olfactory bulb (OB), the role of the kainate subtype of glutamate receptors is unclear. We used immunocytochemical, electrophysiological, and molecular techniques to examine kainate receptor expression and function. Immunocytochemical analyses revealed kainate receptor expression by mitral/tufted (M/T) cells and interneurons, with a subcellular compartmentalization in both culture and brain slices. Co-localization of kainate receptors and synapsin was evident in culture and in the external plexiform layer of brain slices. These results suggest that kainate receptors may participate in dendro-dendritic synaptic transmission. In addition, RT-PCR analysis revealed expression of all kainate receptor subunits (GluR5, 6 and 7 and KA1, 2), and subunit composition can dramatically affect receptor properties. Functional expression of kainate receptors on M/T cells and interneurons was determined by voltage-clamp analyses. Our results suggest that the fast postsynaptic component of excitatory transmission may involve both kainate and AMPA receptors. Furthermore, the immunocytochemical identification of kainate receptors on M/T-cell secondary dendrites suggests a potential presynaptic role. At a low concentration (1 micromolar), kainate also increased spontaneous and evoked excitatory synaptic activity in interneurons; whether this effect is mediated presynaptically and/or postsynaptically remains undetermined. Thus, kainate receptors may play a key role in OB excitatory transmission. Supported by NIH.

MECHANISM OF GLUTAMATE EXCITATION OF THE SOMA AND PROXIMAL DENDRITE OF MITRAL CELLS

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Controversy surrounds the mechanism of glutamate excitation of the somatodendritic membrane of mitral cells in the olfactory bulb. One view proposes that glutamate released from a mitral cell activates autoreceptors by spillover transmission. Another theory postulates glutamate transmission via novel excitatory dendrodendritic synapses linking mitral to granule cells. These synapses are thought to segregate NMDA receptors into an AP5-sensitive pool on granule cells, and an AP5-insensitive pool on mitral cells. Such synapses imply more complex circuitry for odor information processing in the bulb. We studied this issue in rat bulb slices by probing glutamate receptors on mitral cells by laser flash photolysis of 7-nitroindolyl-caged glutamate, a new caged precursor without pre-photolysis activity at glutamate receptors. With GABA-A receptors blocked, currents activated by uncaging glutamate on the mitral soma, primary and secondary dendrites resolved into a fast NBQX-sensitive AMPA-component, and a slow AP5-sensitive NMDA-component. The glutamate responses were strongly attenuated by 50-100 μ M AP5, but not attenuated by 10 μ M BAPTA-AM, a membrane-permeant fast Ca^{2+} buffer. Dendrodendritic feedback currents evoked by voltage pulses were substantially attenuated by BAPTA-AM, demonstrating it was effective at inhibiting transmitter release. This suggests that the glutamate responses evoked by uncaging may not involve transmitter release, and that AP5-sensitive NMDA receptors may occur on the mitral cell membrane. These results are not easily accommodated by the proposed model of excitatory dendrodendritic transmission, and further critical analysis of this issue is warranted. Supported by RO1 NIH-DC04208-01.

IONOTROPIC GLUTAMATE RECEPTOR ACTIVATION SELECTIVELY DEPLETES GABA LEVELS IN ZEBRAFISH OLFACTORY BULB

Michel W.C.¹, Edwards J.G.¹ ¹*Physiology, University of Utah, Salt Lake City, UT*

We previously described a single type of projection neuron, the mitral cell, and 3 types of interneurons in the zebrafish olfactory bulb. The 3 interneurons include tyrosine hydroxylase positive (TH^+) cells and juxtglomerular cells (JG) located in the mitral cell/glomerular layer and granule cells located in the central granule cell layer. Each of the interneurons contains high GABA levels and is thought to be inhibitory. Glutamate acting thru ionotropic glutamate receptors (iGluRs) is responsible for activating each of these neuron types. We used activity dependent labeling to explore iGluR mediated changes in neurotransmitter levels in each neuron type. Isolated olfactory bulbs were incubated for 10 min in ACSF containing the ion channel permeant probe agmatine (AGB, 5 mM) and the appropriate combination of iGluR agonists and antagonists needed to specifically activate only NMDA, AMPA or KA specific iGluR subtypes. Fixed bulbs were embedded in plastic and consecutive 50-100 nm thick sections were stained for glutamate, GABA, TH and AGB immunoreactivity. Glutamate levels were largely unaffected by iGluR activation. No changes in JG cell GABA levels were noted following stimulation with 37-3000 μ M NMDA, however, GABA levels in a subset of granule cells were significantly reduced by 337-3000 μ M NMDA stimulation. GABA depletion is correlated with iGluR activation, as gauged by AGB immunoreactivity levels. Changes in transmitter levels following AMPA and KA receptor activation is being analyzed. Whether odorants elicit similar changes in GABA concentration remains to be determined. Supported by DC01418 and NS07938.

PHARMACOLOGICAL CHARACTERIZATION OF IONOTROPIC GLUTAMATE RECEPTORS IN THE ZEBRAFISH OLFACTORY BULB

Edwards J.G.¹, Michel W.C.¹ ¹*Physiology, University of Utah, Salt Lake City, UT*

Previously, we showed that all odor stimulated labeling of olfactory bulb neurons could be blocked by a mixture of NMDA and AMPA/KA specific ionotropic glutamate receptor (iGluR) antagonists. Application of an AMPA/KA receptor antagonist alone completely blocked labeling in granule cells and tyrosine hydroxylase positive (TH^+) cells but only partially blocked labeling of mitral cells and juxtglomerular (JG) cells. A NMDA receptor antagonist partially blocked labeling of all these cell types. To understand this diversity of antagonist effects we investigated the relative sensitivity of bulbar neurons to iGluR agonists using activity dependent labeling techniques. The technique uses a cation channel permeant probe, agmatine (AGB), to label active bulbar neurons, which are visualized using standard immunocytochemical techniques. After isolated OBs were incubated for 10 min with NMDA (37-3000 μ M), AGB (5mM), and CNQX (50 μ M, AMPA/KA antagonist) the sensitivity of each cell type to NMDA was estimated from the agonist dose eliciting a half-maximal response (EC_{50}). The calculated EC_{50} s for NMDA stimulated mitral cells, granule cells and TH^+ /JG cells were 75, 65 and 80 μ M, respectively. TH^+ /JG cells were pooled because TH immunoreactivity was lost following stimulation with high agonist concentrations. Preliminary KA agonist experiments revealed that most, but not all, mitral cells express KA receptors. In light of the similarities in NMDA receptor sensitivity we anticipate differences in the sensitivity to AMPA and KA receptor agonists may be responsible for the diversity of antagonist effects previously noted. Funded by NIH grants DC01418 and NS07938.

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METABOTROPIC GLUTAMATE RECEPTOR MGLUR1 DIRECTLY AND POTENTLY ACTIVATES MITRAL CELLS IN MAIN OLFACTORY BULB SLICESHeinbockel T.¹, Ennis M.¹ ¹*Anatomy & Neurobiology, University of Maryland School of Medicine, Baltimore, MD*

Mitral cells (MCs), key output neurons of the main olfactory bulb (MOB), express high levels of the Group I metabotropic glutamate receptor, mGluR1. In the present study, we characterized the effects of mGluR agonists/antagonists on the excitability of MCs in rat and mouse MOB slices. Application of nonselective mGluR antagonists (MCPG, LY341495) decreased spike generation and modestly hyperpolarized MCs. These effects persisted in blockers of fast synaptic transmission (CNQX, AP5, gabazine) and were primarily due to a negative shift in the upstate potential generated by MC intrinsic membrane bistability. mGluR antagonists also substantially reduced olfactory nerve-evoked spiking in MCs. Application of the nonselective Group I/II agonist, trans-ACPD, or the preferential Group I agonist DHPG potently and reversibly depolarized MCs and increased their firing rate in a dose-dependent manner. These effects persisted undiminished in the presence of fast synaptic blockers, indicating that they are due to direct activation of mGluRs on MCs. MC excitatory responses to ACPD and DHPG were absent in mGluR1 knockout mice, but persisted in mGluR5 knockout mice. These results indicate that excitatory responses of MCs to Group I/II mGluR agonists are primarily if not entirely, mediated by the mGluR1 subtype. Taken together, our findings suggest that endogenous, synaptically-released glutamate tonically modulates MC excitability via activation of mGluR1. The cellular mechanism(s) via which activation of mGluR1 depolarizes MCs is currently under study. Support PHS grants: DC03195, DC02588, DC00347 & NS36940

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EXPRESSION OF SEROTONIN RECEPTORS IN RAT TASTE RECEPTOR CELLS.Kaya N.¹, Shen T.¹, Herness M.S.¹ ¹*Oral Biology, Ohio State University, Columbus, OH*

Our laboratory has demonstrated that rat taste receptor cells (TRCs) respond to serotonergic stimulation with inhibitions of calcium-activated potassium and sodium currents, possibly via 5HT_{1A} receptors. Others have demonstrated that many TRCs are serotonergic. We hypothesize that serotonergic transmission may play a paracrine role within the taste bud. Actions of 5HT are mediated through seven major receptor families. In our present study, we screened these receptor subtypes to determine their presence in taste buds using RT-PCR performed on RNA extracted from pure populations of rat circumvallate and foliate taste buds with 14 gene specific primers representing receptor subtypes in families 5HT₁ through 5HT₇. Our results suggest that the 5HT_{1A} and 5HT₃ receptor subtypes are expressed in taste receptor cells. To confirm cellular localization, immunocytochemistry with 5HT_{1A} specific primary antibody was performed using FITC immunofluorescence. Typically, taste buds displayed several immunoreactive cells though not all taste buds displayed positive cells. In an ICC:ICC double-labeling experiment, serotonin positive cells and 5HT_{1A} positive cells were observed exclusively in different cell populations, supporting the notion of paracrine processing. Supported by NIH DC00401 and NSF IBN9724062

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GABAERGIC CELLS IN THE GOLDFISH VAGAL LOBEAnderson K.¹, Böttger B.¹, Lariviere K.², Trudeau V.², Finger T.E.¹ ¹*Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO;* ²*Dept. Biology, Univ. Ottawa, Ottawa, Ontario, Canada*

The vagal lobe is the primary sensory nucleus for vagal taste in the brainstem of goldfish. Unlike its counterpart in mammals (the nuc. of the solitary tract), the vagal lobe is laminated, facilitating cellular analysis of connectivity and neurotransmitters within the nucleus. In order to determine the distribution of GABAergic neurons, we utilized immunocytochemistry (ICC) for GABA and in situ hybridization (ISH) for glutamate decarboxylase (GAD), the synthetic enzyme for GABA. We utilized probes for the goldfish form of both GAD65 and GAD67; these gave essentially identical results. Both GAD ISH and GABA ICC label somata of GABAergic neurons and results from the two methods were similar. GABAergic somata are especially prevalent in the lower part of layer II and in layer XI of the sensory zone. Roughly half of the neurons in these layers are GABAergic. Additional GABAergic somata are situated in sensory layers V, VII, and IX. The majority of neurons in the sensory layers have radially-directed dendrites entering the layers of primary afferent termination and are therefore potentially second-order gustatory neurons. In addition, small interneurons of the motor layer reacted with the GABA probes. Supported by NIH grant DC 00147 to T.E.F.; NSERC Grant to V.T. and NSERC graduate scholarship to K.L.

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CCK-8 POTENTIATES THE SYNAPTIC RESPONSE TO AFFERENT STIMULATION IN THE PRIMARY GUSTATORY NUCLEUS OF GOLDFISHSharp A.A.¹, Finger T.E.¹ ¹*Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO*

We have used an in vitro slice preparation of the goldfish primary gustatory nucleus, the vagal lobes, to demonstrate that primary gustatory afferents utilize glutamate as their primary transmitter acting via both AMPA and NMDA receptors (Smeraski et al., Chem. Senses, 1999). Further, we have shown that the amplitude of this synapse can be modulated by GABA (Sharp and Finger, submitted). Immunohistochemical analysis of the vagal lobes has revealed the presence of the neuromodulator cholecystokinin (CCK) within the sensory layers of the lobe (Farrell et al. AChemS 2001). Since CCK affects appetite and food intake, we wanted to determine whether CCK modulates transmission at the primary gustatory afferent synapse.

The in vitro slice preparation of the vagal lobe permits recording synaptic field potentials generated by second-order neurons in response to stimulation of the incoming gustatory nerves. This field EPSP is a measure of the synaptic efficacy of the primary afferent synapse. Bath application of the bioactive, sulfated octapeptide fragment of CCK (CCK-8S, 1 μ M) produces a 10-30% increase in the amplitude of the afferent evoked field EPSP. Application of CCK-8S in the presence of the NMDA channel antagonist APV also potentiates the field EPSP. This indicates that CCK-8S likely increases the release of glutamate from the primary gustatory afferent terminals without requiring an increase of the NMDA-mediated currents in the second order neurons. Supported by NIH grant DC00147 to T. Finger.

INVESTIGATION OF THE SIGNAL TRANSDUCTION PATHWAY IN VOMERONASAL RECEPTOR NEURONS OF THE RAT

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Sensory neurons of the vomeronasal organ (VNO) detect volatile chemicals that are released by conspecific animals and convey information for social and reproductive behavior. The signal transduction pathway in the vomeronasal receptor neurons (VRNs) is not known, but is distinct from that of the sensory neurons of the main olfactory system. Using Ca²⁺ imaging and electrophysiological recordings we investigated the signal transduction pathway of urine perception in freshly dissociated rat VNO neurons. We found that application of urine induced a transient increase in intracellular Ca²⁺ that was dependent on the activity of phospholipase C and diacylglycerol lipase. The Ca²⁺ transient was not dependent on depletion of intracellular Ca²⁺ stores but on the presence of extracellular Ca²⁺. The urine response was not sensitive to modulators of adenylyl cyclase and inhibitors of inositol 1,4,5-trisphosphate receptors. Application of polyunsaturated fatty acids (linolenic acid and arachidonic acid, synthesized in living cells from diacylglycerol) induced Ca²⁺ transients in FURA2 measurements and inward currents in whole-cell voltage-clamp recordings. Pharmacological inhibition of lipoxygenase and cyclooxygenase induced a transient increase in intracellular Ca²⁺, possibly by increasing the endogenous level of polyunsaturated fatty acids, leading to activation of transduction channels. These data provide some new insight in the signal transduction pathway of rat VRNs.

NETRIN-1 REGULATES THE MIGRATION OF LHRH NEURONS TO THE BASAL FOREBRAIN

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Luteinizing hormone-releasing hormone (LHRH) neurons migrate from the vomeronasal organ (VNO) to the forebrain. In mice, LHRH neuron migration is guided by the caudal branch of the vomeronasal nerve (cVNN). Cues that regulate the trajectories of the cVNN are candidates for determinants of the final destination of LHRH neurons. We have previously shown that deleted in colorectal cancer (DCC) plays an important role in regulating the final destination and fate of LHRH neurons in mice. In DCC^{-/-} mice, less than 10% of the normal number of LHRH neurons is found in the basal forebrain and many LHRH neurons are displaced into the cerebral cortex. These results suggested that netrin-1 regulates the trajectories of DCC⁺ axons that act as guides for the migration of LHRH neurons. However, other netrin-1 receptors such as unc5h3 are also expressed in the olfactory system could also play a role in migration of these neurons. We therefore examined the trajectory of the cVNN and the migration of LHRH neurons in netrin-1^{-/-} mice. Compared to wild-type mice, the majority of cVNN fibers turn aberrantly in netrin-1^{-/-} mice. At E15, there are about twice as many LHRH neurons in the cortex of mutant mice compared to wild-type mice. The ratio of LHRH neurons in the dorsal forebrain compared to ventral forebrain in netrin-1^{-/-} mice is nearly 3 to 1 at E15 compared to a 1:1 ratio in wild-type littermates. These data are consistent with the idea that loss of netrin-1 function results in aberrant cVNN trajectories and the migration of many LHRH neurons to inappropriate destinations. (Supported by NIH grant DC00953)

IMMUNOHISTOCHEMISTRY OF THE VOMERONASAL ORGAN IN CALLITRICHIDS AND PROSIMIANS: AN ONTOGENETIC STUDY

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Numerous studies of the rodent vomeronasal organ (VNO) postnatal ontogeny exist but few studies have addressed the primate VNO. We used immunohistochemistry to assess VNO sensory neuron distribution in four callitrichids and two prosimians. These included three tamarins (*S. geoffroyi*, *L. rosalia*, and *L. chrysomelas*), a marmoset (*C. jacchus*), and the prosimians *E. mongoz* and *O. garretti*. Bipolar, neuron specific beta tubulin (BT+) cells were sparse in neonatal (especially *S. geoffroyi*) and adults of both tamarin genera. Qualitatively, a greater density of bipolar BT+ cells was observed in juveniles of both genera. Patches of BT+ cells were separated by non-sensory epithelium in *S. geoffroyi* whereas sensory cells surrounded the lumen of adult *C. jacchus* VNO. In contrast, regions of *L. rosalia* resembled *E. mongoz* and *O. garretti* with BT+ cells segregated on the medial side of the VNO. These findings suggest that VNO neurosensory organization varies more in callitrichids compared to other primate taxa. Further, our observations are consistent with perinatal VNO function in some callitrichid species (*L. rosalia*) but not others (*S. geoffroyi*). Supported in part by ONDCP #N66001 to EEM.

ASSESSMENT OF OLFACTORY FUNCTION AND ANDROSTENONE ODOR THRESHOLDS IN MAN WITH OR WITHOUT COVERING THE VOMERONASAL DUCT

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The functionality of the human vomeronasal organ (VNO) is a matter of debate. In own studies we demonstrated that a vomeronasal duct (VND) was detected in approximately 50% of the subjects. Similar numbers of adults are able to perceive androstenone odor which has been addressed as a "human pheromone". Considering that the effect of these putative "pheromones" is often used in the context of activation of the VNO the aim of this study was to look at thresholds for androstenone in adults with or without covering of the VND. In addition, the study aimed to look for correlations between sensitivity to androstenone odor and general olfactory function. A total of 34 subjects (19 men, 15 women, age 18-78 years) participated. In addition to androstenone odor thresholds (concentration 0.1uM to 10mM) the subjects' general olfactory abilities were measured using the "Sniffin' Sticks" test battery which consists of tests for odor identification, odor discrimination, and phenyl ethyl alcohol odor threshold. Measurements were performed w/o covering the VND. In each subject tests were performed on one side only. As established with the olfactory test battery 6% of the subjects were anosmic; however, 24 % of the subjects had a specific anosmia for androstenone. Covering the VND had no effect on olfactory test results or androstenone thresholds. Compared to men, female subjects had higher olfactory sensitivity and lower androstenone thresholds. In addition, olfactory test scores were found to correlate with androstenone thresholds. These results indicate that the presence of a VND does not play a major role in the perception of androstenone odor and other odors.

EXPRESSION OF SECOND MESSENGER PATHWAYS IN THE VOMERONASAL ORGAN

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The vomeronasal organ (VNO) is the receptor portion of the accessory olfactory system and transduces chemical cues that identify social hierarchy, reproductive status, conspecifics, and prey. Signal transduction in VNO neurons may be accomplished via an inositol 1,4,5- trisphosphate (IP₃)-activated calcium conductance that includes a different set of G-proteins than those identified in vertebrate olfactory sensory neurons. We used immunohistochemistry (IHC) and SDS-PAGE to localize type-specific IP₃ receptor (IP₃R) proteins in rat VNO epithelium. IP₃R1 expression was weak or absent. Antisera for IP₃R2 and IP₃R3 recognized appropriate MW proteins by SDS-PAGE and labeled protein could be abolished by preabsorption of respective antibody with antigenic peptide. In tissue sections, IP₃R2 immunoreactivity was present in the supporting cell zone but not in the sensory cell zone. IP₃R3 immunoreactivity was present throughout the sensory zone and overlapped, in the microvillar layer, that of TRP2 (transient receptor potential channel 2). Co-immunoprecipitation of IP₃R3 and TRP2 from VNO lysates confirmed the overlapping immunoreactivity patterns. The protein-protein interaction complex between IP₃R3 and TRP2 could initiate calcium signaling leading to electrical signal production in VNO neurons. We are currently exploring whether this interaction is direct or indirect via scaffolding proteins recognizing PDZ domain interaction motifs. Supported by FAA grant 01-6-022 to E.E.M. and T32-DC00044 to J.H.B.

PEROMONAL ACTIVATION OF VOMERONASAL NEURONS IN PLETHODONTID SALAMANDERS

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Pheromones from the mental glands of male plethodontid salamanders increase sexual receptivity in conspecific females. The pheromone enters the vomeronasal organ during courtship to produce this effect. Vomeronasal neurons from female *Plethodon shermani* were examined following exposure to male pheromone or saline placed on the nares. Agmatine was used in conjunction with the pheromone to enable immunocytochemical visualization of chemosensory neurons that were activated by the pheromone. Olfactory neurons exposed to pheromone or saline, and vomeronasal neurons exposed to saline did not demonstrate significant labeling. A population of vomeronasal neurons was intensely labeled following exposure to the pheromone. These labeled neurons were distributed diffusely throughout the entire vomeronasal epithelium but were most concentrated in the caudal sensory epithelium. This study demonstrates that a specific population of vomeronasal neurons in a female plethodontid salamander is responsible for transmitting pheromonal information to the brain to produce modifications in behavior. Supported by the National Science Foundation IBN-0110666.

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DATES OF FUTURE MEETINGS

April 9 - 13, 2003

April 21 - 25, 2004

April 13 - 17, 2005

ACHEMS XXI ABSTRACTS

BOOK ERRATA

Despite the best efforts of your Program Committee led by the Program Chair, Alan Spector, working with COS, the following abstracts printed out from electronic copy with incorrect greek symbols. Dr. Spector, COS and the ACHemS Program Committee are in no way responsible for this error. Hence, this addendum to your conference's Book of Abstracts. This will in no way affect the published version of the abstracts which will appear in a special issue of the journal *Chemical Senses*.

Welcome to Sarasota, and enjoy the meeting!

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Slide

Functional Consequences of Manipulating
Chemosensory Systems

SIGNAL TRANSDUCTION OF UMAMI TASTE BY α -GUSTDUCIN AND α -TRANSDUCIN

He W.¹, Margolskee R.F.², Damak S.¹ ¹*Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY;* ²*Howard Hughes Medical Institute, New York, NY*

The transduction events underlying umami taste, elicited by monosodium glutamate (MSG), are poorly understood. One G-protein coupled receptor, taste mGluR4, has been identified in taste cells and implicated in umami taste. To determine if α -gustducin might be involved in umami responses, we carried out two bottle preference (TBP) tests comparing α -gustducin knockout (KO) with wild-type mice. We found that α -gustducin KO mice showed a diminished response to MSG. We hypothesized that the residual response of these mice might be carried by α -transducin (also expressed in taste tissue). We carried out TBP tests comparing α -gustducin and α -transducin single and double KO and WT mice. Concentrations of MSG between 10 and 300 mM were preferred by WT and α -transducin KO mice and less preferred by α -gustducin KO mice, whereas the double KO animals were indifferent to these concentrations. IMP concentrations between 5 mM and 100 mM were preferred by WT and α -transducin KO mice, whereas α -gustducin KO and double KO mice were indifferent to the compound at these concentrations. With high concentrations of IMP or MSG (aversive to WT mice), there was little difference between the four groups. These data indicate that α -transducin and α -gustducin are involved in the transduction of umami preference. MSG signals appear to be transduced via both G proteins, whereas IMP signals are transduced only via α -gustducin. Supported by NIH grants DC03055 and DC3155 (RFM), and DC04766 (SD). RFM is an Investigator of the Howard Hughes Medical Institute.

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Poster

Disease and Clinical Conditions

THE MOLECULAR GENETICS OF HUMAN CONGENITAL GENERAL ANOSMIA

Feldmesser E.¹, Halbertal S.², Frydman M.³, Gross-Isseroff R.¹, Lancet D.¹ ¹*Dept. of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel;* ²*Dept. of Otorhinolaryngology, Kaplan Hospital, Rehovot, Israel;* ³*Dept. of Genetics, Sheba Medical Center, Tel Hashomer, Israel*

While the genetics of blindness and deafness has been thoroughly investigated, much less is known about smell blindness - Congenital General Anosmia (CGA), except for the X-linked Kallmann Syndrome form. Through newspaper advertisement and a preliminary questionnaire that eliminated acquired anosmia cases, we obtained 162 presumed CGA subjects (~1/10,000 incidence). Of these, 62 were familial, in 24 families, a prevalence broadly consistent with autosomal recessive transmission. The largest family had 6 anosmics in 3 generations. A second family, with 3 CGA siblings, suggested putative non-Kallmann X-linked inheritance. A candidate gene table was constructed from genome data, based on animal models and on the notion that CGA is caused by genes affecting overall olfactory function. These include the cyclic nucleotide-gated channel alpha chain (*CNGA2*, Chr X), the olfactory G α protein (*GNAL*, Chr 18), adenylyl cyclase III (*ADY3*, Chr 2), and the transcription factor Olf1 (*EBF*, Chr 5). Homozygosity mapping for all CGA patients and segregating haplotype analysis in families are used as tools for causative gene identification. Short Tandem Repeat and high throughput mass-spectrometry-based Single Nucleotide Polymorphisms (SNP) analyses are employed for genetic linkage and association studies, to help decipher the molecular underpinnings of this chemosensory dysfunction. Supported by NIH (DC00305), the Crown Human Genome Center and the Israeli Ministry of Science.

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Poster

Neural Coding in the Peripheral Olfactory
System

DISRUPTION OF GAP JUNCTIONS IN OLFACTORY NEURONS ALTERS OLFACTORY RESPONSES TO SOME ODORS

Zhang C.¹, Restrepo D.¹ ¹*Department of Cellular and Structural Biology, Neuroscience Program and the Rocky Mountain Smell and Taste Center, University of Colorado Health Sciences Center, Denver, CO*

One of the challenging questions in understanding olfactory transduction is whether olfactory information is modulated in the primary olfactory pathway before transmission to the olfactory bulb. Our studies have demonstrated spatial distribution and expression of multiple gap junction protein subunits (connexins) in the olfactory epithelium. We hypothesize that gap junctional communication between olfactory neurons modulates peripheral olfactory coding. To test this hypothesis, we have designed and generated a dominant negative connexin 43 (Cx43) mouse (DNCX). The transgene includes a 0.9kb mouse olfactory marker protein (OMP) promoter that drives the expression of Cx43/ β -galactosidase fusion protein (Cx43/ β -gal). Cx43/ β -gal inactivates Cx43 channels by competing with endogenous Cx43 during assembly. Integration of the transgene was confirmed by Southern analysis. *In situ* hybridization showed that Cx43/LacZ mRNA was expressed in the olfactory epithelium and vomeronasal organs. The expression pattern was consistent with expression directed by the OMP promoter. Western analysis indicated the presence of Cx43/ β -gal in the olfactory epithelium. The ratio of electroolfactogram responses to octanal/benzaldehyde ($p < 0.001$, $n = 25$) and octanal/cineole ($p < 0.05$, $n = 19$), but not the ratio of cineole/benzaldehyde, were reduced in DNCX. Our study suggests that gap junctions are involved in modulating olfactory activity in some olfactory receptor neurons. This work was supported by grants DC00566 and DC04657 from the NIDCD.

CHEMOKINE-MEDIATED INFILTRATION OF MACROPHAGES INTO THE OLFACTORY EPITHELIUM FOLLOWING TARGET ABLATION

Getchell T.V.¹, Subhedar N.¹, Shah D.S.¹, Hackley G.¹, Partin J.V.¹, Sen G.¹, Getchell M.L.¹ ¹*Sanders Brown Ctr. on Aging, U. of Kentucky, Lexington, KY*

The C-C subfamily of chemokines are small bioactive proteins whose activities are mediated by binding to target cell receptors that belong to a family of G protein-coupled receptors. We investigated the expression of two C-C chemokines, macrophage inflammatory protein- α (MIP-1 α) and monocyte chemoattractant protein (MCP-1), and their cognate receptors CCR1 and CCR2 respectively, in the murine olfactory epithelium (OE) following olfactory bulbectomy (OBX). Using ELISA, there was a transient upregulation of both chemokines from at or below the limit of detection (MIP-1 α , 1.5 pg/ml; MCP-1, 9 pg/ml); MIP-1 α protein peaked at 4.4 ng/ml at 3 d post-OBX, and MCP-1 at 42 pg/ml at 16 hr post-OBX. Using relative quantitative RT-PCR, a transient upregulation of mRNAs for the chemokines and their receptors peaked at 2-3 d post-OBX. Digoxigenin-labeled MIP-1 α , MCP-1, CCR1 and CCR2 probes transcribed from RT-PCR products were synthesized for non-isotopic *in situ* hybridization. There was a systematic increase in the numbers of mRNA⁺ cells in the OE that peaked at 16 hr post-OBX; adjacent tissue sections stained with CD68 and F4/80 antibodies suggested that the mRNA⁺ cells were resident and infiltrating macrophages. Our studies indicate that the chemokines MIP-1 α and MCP-1, signaling through CCR1 and CCR2 respectively, participate in intercellular signaling mechanisms by which the degeneration of olfactory neurons is coupled with phagocytosis and the proliferation of progenitor cells leading to neurogenesis and regeneration of the OE. Supported by NIH-NIA R01 AG-16824-21 (TVG)

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BACK-PROPAGATING ACTION POTENTIALS ACTIVATE DIFFERENTIAL SPATIAL CALCIUM RESPONSES IN MITRAL CELLS IN RAT OLFACTORY BULB SLICES

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The aim of this study was to compare back-propagating action potential (BAP) activated Ca²⁺ responses in mitral cell soma, primary dendritic trunk and tuft. The study was carried out on rat olfactory bulb slices; methods were based on custom-made two-photon microscopy and patch clamp techniques, measuring fluorescence with Oregon Green. BAP elicited the smallest Ca²⁺ increase in mitral cell somas ($\delta F/F$, 6.9% \pm 1.1). The Ca²⁺ increases in proximal, middle and distal dendritic trunk were approximately 21.0%, 27.6% and 21.3%, respectively, with no significant difference (student *t* test). The increases in the proximal tuft (from branching level I to IV, starting from the origin of the tuft) were approximately 45.0%, 39.2%, 52.0% and 43.7%, respectively, with no significant differences. Significant differences were present between soma and primary dendritic trunk ($P < 0.0005$), between primary dendritic trunk and the proximal glomerular tuft branching levels I-IV ($P < 0.01$), and between tuft levels I-IV and more distal tuft branches (21.1% \pm 3.0) ($P < 0.005$). These results suggest that the rat mitral cell has at least four Ca²⁺ compartments: soma, primary dendrite trunk, proximal glomerular branches (I to IV) and distal glomerular branches (> IV). We speculate Ca²⁺ fluxes play different roles in each of these compartments in controlling transmitter release, membrane excitability through Ca²⁺-activated K⁺ channels, and neuronal plasticity. Supported by NIDCD, Human Brain Project and MURI grant.

ADAPTOR PROTEINS MODULATE PROTEIN-PROTEIN INTERACTIONS AND BIOPHYSICAL PROPERTIES OF AN OLFACTORY BULB K⁺ CHANNEL

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Two adaptor proteins expressed in the olfactory bulb differentially modulate v-Src-induced Kv1.3 (Shaker family K⁺ channel) phosphorylation and modulation of channel function via alteration of SH2- and SH3-mediated protein-protein interactions. Grb10 adaptor significantly reduces v-Src-induced Kv1.3 phosphorylation, whereas tyrosine phosphorylation of Kv1.3 is modestly increased in the presence of n-Shc adaptor. The proline rich sequences contained in Grb10 adaptor protein may compete for the SH3 domain of Src, to decrease the ability for Src to phosphorylate Kv1.3 and suppress Kv1.3 current magnitude, increase the inactivation time constant (τ_{inact}), and disrupt cumulative inactivation during repetitive voltage stimulation. N-Shc adaptor also acts to prevent the v-Src-induced increase in the Kv1.3 τ_{inact} but additionally reverses a shift in voltage-dependence typically observed for Kv1.3 in the presence of v-Src. Through site-directed mutagenesis of the regulatory Tyr residues in the CH domain of n-Shc and comparison of the activity of n-Shc with a close family member Sck, data suggest that a portion of the CH domain that includes tyr 221, 222 and 304 and is homologous to Sck regulates a shift in Kv1.3 voltage-dependence and inactivation kinetics. Collectively these data indicate that the repertoire of adaptor molecules expressed in olfactory bulb or other neurons and the specific capacity for regulating the proximity of kinase to its effector (the ion channel) may influence the electrical phenotype of a neuron via phosphorylation. Supported by NIH R29DC03387.

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FUNCTIONAL AND BIOCHEMICAL DIFFERENCES BETWEEN MICROVILLAR AND CILIATED OLFACTORY RECEPTOR NEURONS IN CATFISH

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The olfactory system of vertebrates encompasses ciliated (cORN) and microvillar receptor neurons (mvORN) projecting to different territories in the extended olfactory bulb (OB). The cORNs and mvORNs utilize different families of receptor molecules (OR, V1R, V2R) coupled to different G-proteins. In fishes, unlike rodents, the different morphological types of receptor cells are intermingled in a single sensory epithelium but nonetheless project to different territories in the OB. We used anatomical, molecular and electrophysiological methods to test whether in catfish a correlation exists between the morphology of the ORN, the molecular transduction system and the type of odorant detected. Electrophysiological tests with 3 types of biologically relevant stimuli revealed that ORNs detecting bile salts project to the medial middle part of the OB. Retrograde Dil tracing from this area identified these as cORNs which utilize G α_{olf} . Likewise, recordings from the anterior dorsal and the ventral side of the OB showed that these areas mainly process amino acid odor information and receive input from both mvORNs and cORNs. Nucleotide responses are present in the posterior dorsal part of the OB which receives input from mvORNs. Our experiments suggest that two different types of ORNs (ciliated and microvillar) detect different classes of odorants: bile salts by cORNs and nucleotides by mvORNs. Supported by NIH Grant DC03792 (J.C., P.I.)

DIFFERENTIAL RESPONSIVENESS OF CILIATED AND MICROVILLAR OLFACTORY RECEPTOR NEURONS IN GOLDFISH

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Although odor information in fish is detected by at least 3 different types of olfactory receptor neurons (ORNs) (ciliated, microvillar and crypt), little is known about the specificities of these cell types. We explored this question in goldfish by means of correlated histological and electrophysiological approaches. Immunohistochemical studies found ciliated ORNs expressing G_{olf} distributed across the entire surface of the sensory epithelium including both the regions adjacent to the raphe (peri-raphe) and the outer regions (marginal) of the lamellae. In contrast, microvillar and crypt ORNs expressing either G_{ao} or G_{aq} were concentrated in peri-raphe regions. Multi-unit in vivo recordings found all regions of all lamellae to be consistently sensitive to amino acid stimuli (74 of 74 loci). In contrast, ORNs responding to identified sex pheromones (17,20 β -dihydroxyprogesterone and/or 15-ketoprostaglandinF₂ α) were plentiful in peri-raphe regions, but were relatively uncommon (<20% of loci) in the marginal regions where ciliated ORNs predominate. In conclusion, the distribution of ORNs that respond to sex pheromones suggests that microvillar and/or crypt ORNs mediate responses to these cues while some ciliated ORNs likely respond to amino acids alone. Supported by NIH/DC03792; NSF/IBN9723798

EXPERIMENTAL EVIDENCE THAT 7 α , 12 α , 24-TRIHYDROXY-5 α -CHOLAN-3-ONE 24-SULFATE FUNCTIONS AS A SEA LAMPREY SEX PHEROMONE

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Recently it has been found that male sea lamprey (*Petromyzon marinus*) release a potent sex pheromone, 7 α , 12 α , 24-trihydroxy-5 α -cholan-3-one 24-sulfate (3-keto petromyzonol sulfate) upon spermiation. This pheromone appears to induce preference behavior and locomotion in ovulating females under controlled laboratory conditions. It has yet to be demonstrated that a synthetic copy of 3-keto petromyzonol sulfate stimulates the olfactory organ and induces characteristic behaviors in ovulating females. To provide conclusive evidence that synthetic 3-keto petromyzonol sulfate functions as a sex pheromone in a natural spawning environment, we first used electro-olfactograms (EOG) to determine the detection threshold and dose response curves of the synthetic pheromone. Then in a section of spawning stream we observed the behavioral response of ovulating females to the synthetic pheromone (at concentrations determined by EOG experiments). EOG results showed that the synthetic pheromone is detected at approximately 10⁻¹² M. When introduced into the spawning stream section, ovulating females swam to and stayed at the source of the synthetic pheromone. We conclude that synthetic 3-keto petromyzonol sulfate is able to function as a sex pheromone in a natural environment at concentrations that are likely to be encountered in the wild. To our knowledge this is the first vertebrate study to effectively demonstrate a synthetic pheromone compound in a natural environment. The Great Lakes Fishery Commission supported this study.

DO SOMATOSENSORY TACTILE STIMULI INTERACT WITH TASTE AND AROMA SIGNALS TO MODULATE PERCEPTION?

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Flavour is defined as the combined perception of mouth-feel, texture, taste and aroma. Within the food industry, the ultimate aim is to understand how varying these flavour components affects flavour quality so that foods can be formulated to deliver maximum consumer acceptability when they are eaten. The perception of sweetness and flavour were studied in viscous solutions containing 5g/l sucrose, 100ppm iso-amyl acetate and varying concentrations of three hydrocolloid thickeners (guar gum, λ carrageenan and hydroxypropylmethyl cellulose (HPMC)). Zero-shear viscosity of the samples ranged from 1 – 5000 mPas. Perception of both sweetness and aroma were suppressed at thickener concentrations above c* (critical coil overlap concentration). Sensory data for the three hydrocolloids was not adequately correlated with their concentration relative to c* (c/c* ratio), particularly above c*. However, when perceptual data was plotted against the Kokini oral shear stress (τ), calculated from rheological measurements, data for the three hydrocolloids aligned to form a master-curve, enabling the prediction of flavour intensity in such systems. The fact that oral shear stress can be used to model sweetness and aroma perception supports the hypothesis that somatosensory tactile stimuli can interact with taste and aroma signals to modulate their perception. This work was funded by Firmenich SA Geneva.

PHOSPHOINOSITIDE SIGNALING IN TASTE CELLS

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Inositol 1,4,5 trisphosphate (IP₃) has been implicated in the transduction of bitter compounds, artificial sweeteners, and recently, in umami taste. Of these pathways, the role of IP₃ in bitter transduction is best understood. Bitter compounds bind to the T2R/TRB class of bitter taste receptors, which are coupled to the heterotrimeric G protein gustducin. Alpha-gustducin activates phosphodiesterase (PDE1A) to reduce intracellular cAMP, while its $\beta\gamma$ partners ($\beta\gamma$ 13) activate PLC β 2 to produce IP₃ and diacylglycerol. Although the role of the reduced cAMP is not clear, the IP₃ binds to receptors on the smooth endoplasmic reticulum to release Ca²⁺ from intracellular stores. In collaboration with R. Margolskee's laboratory, we have identified 2 downstream effectors of the bitter signaling cascade: the Type III IP₃ receptor and a store operated channel, most likely Trp-T. Calcium imaging experiments with mudpuppy taste cells and GFP-labeled mouse taste cells expressing gustducin have shown that prolonged stimulation with the bitter stimulus denatonium elicits a transient increase in intracellular Ca²⁺ due to release from internal stores followed by a sustained influx of Ca²⁺. Treatment of taste cells with thapsigargin to deplete internal Ca²⁺ stores mimics the Ca²⁺ influx, suggesting that store-operated channels are present in taste cells and mediate the Ca²⁺ influx. Further experiments will be required to determine if Trp-T is the source of the Ca²⁺ influx.

SENSORY MEASUREMENT OF DYNAMIC FLAVOR PERCEPTION IN ICE CREAM WITH DIFFERENT FAT LEVELS AND FLAVORINGS

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Intro: Flavor compounds vary in physicochemical properties and, therefore behave differently in foods with different fat content. The objective was to investigate differences in dynamic flavor perception in a realistic food system, and relate them to a range of molecular descriptors for flavor compounds. Ice creams with different fat levels (3, 6, and 12% milk fat) and flavoring (β -ionone (berry), δ -nonalactone (coconut), isopentyl acetate (banana), vanillin (vanilla)) were examined. Approximately equi-intense concentrations (in 12% fat) were selected. Samples were analyzed with descriptive analysis and Time-Intensity, evaluating perceived melt rate and flavor intensity (trained panel N=12, 3 replicates). Data were analyzed by ANOVA, Principal Component Analysis (PCA) and Partial Least Squares Regression (PLSR). Results: Descriptive analysis showed large differences in sensory properties for ice creams with different fat levels, and significant effects of fat level on flavor intensity for all four compounds. PCA of Time-Intensity showed faster perceived melt rates, increases and decreases in dynamic flavor perception with lower fat levels. Individual flavor compounds were affected differently by changes in fat level. By PLSR increase and decrease rates of dynamic flavor perception were reliably modeled to a number of molecular descriptors. Grant support: Danish Dairy Research Foundation (Danish Dairy Board) and Danish Government. Flavor donation: Haarmann & Reimer GmbH.

GYMNEMA SYLVESTRE SWEET-BLOCKING EFFICACY ON TONGUE TIP VS. WHOLE MOUTH

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This study investigated differences in the sweet-blocking efficacy of *Gymnema sylvestre* (GS) for different sweeteners (acesulfame K, sucrose, glucose and Na saccharin) when presented to the tongue tip or the whole mouth. Participants assessed the stimuli following pretreatment with GS tea, rating sweeteners solutions (and water) using tongue tip or whole mouth stimulation in different sessions. They indicated if each stimulus was sweet or not sweet, and gave a sureness judgment, from which the R-index, a signal detection measurement, was calculated. Repeated measures ANOVA on R-index values of 12 subjects indicate that there is no significant difference in the sweetness between sweeteners ($p=0.20$), that they are more readily perceived as sweet with stimulation of the whole mouth than with the tongue tip ($p=0.0004$), and that GS blocks sweeteners to different extents ($p=0.02$). All stimuli but saccharin were perceived as being sweeter when presented whole mouth, which showed no significant difference between stimulation conditions (Scheffe's test, $p=0.24$). Only the whole mouth presentations of sucrose, glucose and aspartame were significantly above chance performance ($\alpha=0.05$, Bi & O'Mahony, *J. Sens. Studies*, 1995), indicating that GS blocked sweetness completely for tongue tip stimulation, but not whole mouth stimulation for most sweeteners. Whether this is due simply to the phenomenon of spatial summation (whereby perceived intensity increases with increasing area of stimulation), or due to differences in physiological mechanisms between the tongue tip and the rest of the mouth, remains to be determined. *Self-funded project*

BEHAVIORAL EVIDENCE FOR A ROLE OF GUSTDUCIN IN UMAMI TASTE

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The taste perception of glutamate (MSG) has been termed "umami". A characteristic feature of umami taste is its potentiation by 5'-ribonucleotides. A truncated form of mGluR4 (taste-mGluR4) has been identified in taste cells and implicated as an umami taste receptor. When expressed in heterologous cells, taste-mGluR4 decreases intracellular cAMP, however, the G protein involved has not been identified. α -Gustducin is a G protein α subunit that activates phosphodiesterase (PDE) to decrease intracellular cAMP in taste cells. Gustducin is known to mediate bitter taste, but its role in umami has not been examined. In this study we used standard 2-bottle preference tests on gustducin knockout (KO) and wild type (WT) mice to compare taste preferences for ascending concentrations of MSG and MSG plus 1 mM IMP. Statistical comparisons between KO and WT mice revealed that WT mice strongly preferred 30 and 100 mM MSG to water ($p<0.01$), however, KO mice showed no preference for any concentration of these compounds. Similar results were obtained for MSG plus IMP. Denatonium was used as a control, and as expected, WT mice avoided denatonium significantly more than the KO mice. These data suggest that gustducin may play a role in umami taste transduction. Supported by NIH grants DC03013 to SCK and DC03055 to RFM.

ACTIVATION OF PURINERGIC RECEPTOR SUBTYPES DIFFERENTIALLY MODULATES MOUSE ORN ODOR RESPONSIVENESS

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Extracellular ATP, an important signaling molecule in numerous systems, is released by neurotransmitters, cell damage, and potentially, by noxious odors. Released ATP binds purinergic receptors and stimulates an increase in intracellular calcium. Our previous studies showed that ATP differentially modulates odor responsiveness in ORNs with suppression occurring more frequently than enhancement. Here we determined that odor response suppression or enhancement depends on activation of specific purinergic receptor subtypes. We found that (1) P2Y receptor selective agonists (UTP and ADP- β S) caused suppression of the odor response, where the response of an ORN to co-application is less than the sum of the responses to the individual components, and (2) a P2X receptor selective agonist (β y-MeATP) caused enhancement, in which the response to co-application is larger than the sum of the individual components. A few studies have provided indirect evidence that neurotransmitters modulate odor sensitivity at the level of the ORN. This is the first report with direct evidence that a neuromodulator, ATP, can differentially modulate the odor responsiveness of ORNs. The complement of P2X and/or P2Y receptor subtypes expressed in the ORN will determine whether the odor response is enhanced or inhibited in the presence of ATP. The predominantly suppressive effect of ATP on odor responses could play a role in the reduced odor sensitivity that occurs during acute exposure to noxious fumes and may be a novel neuroprotective mechanism. This research was supported by NIH NIDCD DC04953 and DC02994.