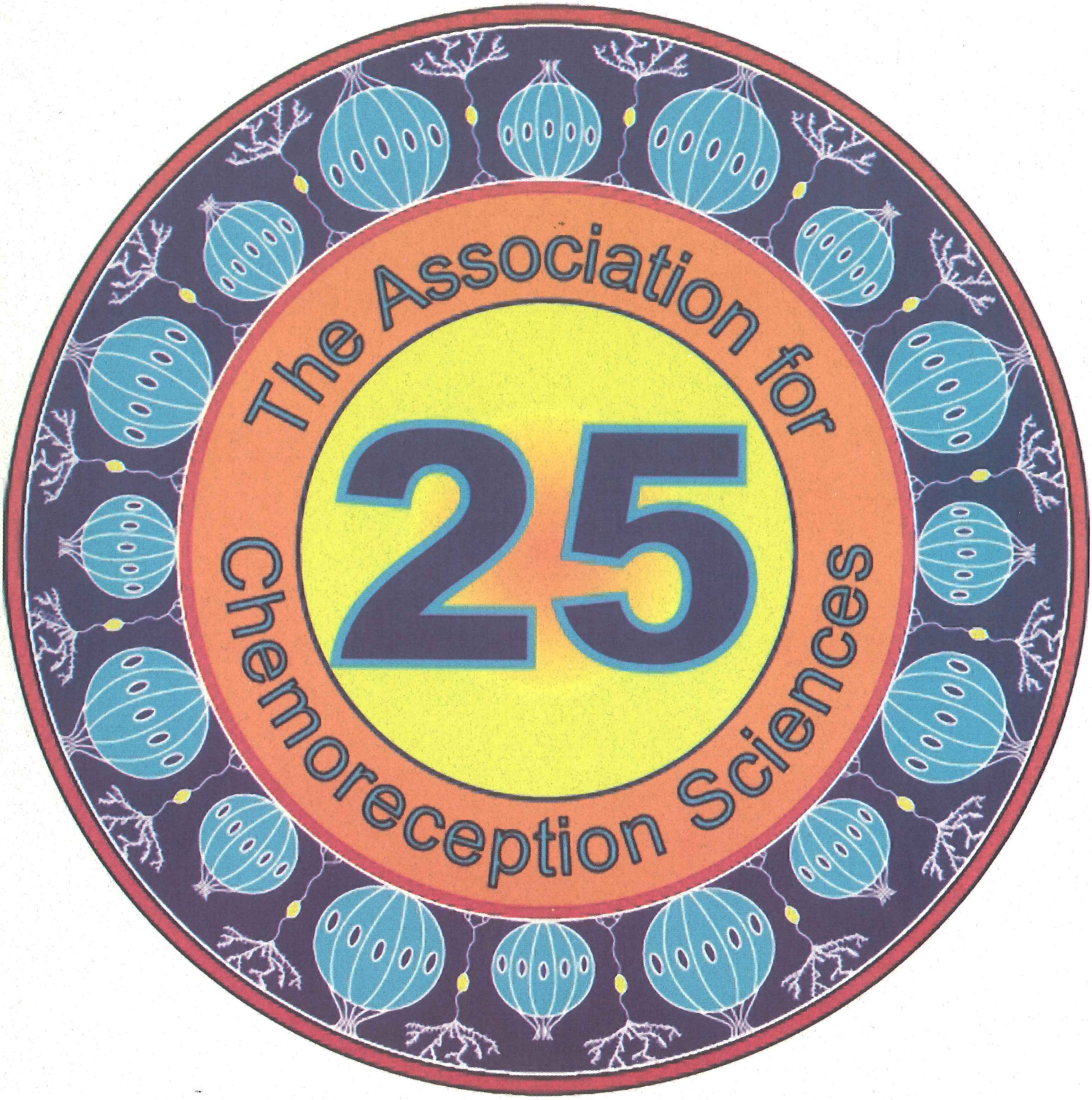
Abstracts



AChemS 25th Annual Meeting

April 9-13, 2003 • Sarasota, Florida

The Association for Chemoreception Sciences appreciates grant support from

The National Institute on Deafness and Other Communication Disorders, National Institutes of Health

AChemS is also grateful for the generous support of its Corporate Sponsors:

Twenty Fifth Annual Givaudan Lectureship
Givaudan Corporation

Eighteenth Annual Takasago Award for Research in Olfaction
Takasago Corporation

Twelfth Annual Moskowitz Jacobs Award for Research in the Psychophysics of Taste and Olfaction
Moskowitz Jacobs, Inc.

Tenth Annual Ajinomoto Award to Promising Young Researcher In the Field of Gustation
Ajinomoto USA

Additional Support for the Symposia and Scientific Program has been generously provided by:
Firmenich, SA
International Flavors and Fragrances, Inc.

Support for the AChemS 25th Anniversary Special Events provided by
Avon Products, Inc.

Exhibitors: **Marcel Dekker Inc, Olympus America Inc, Tucker-Davis Technologies**

* * *

AChemS Executive Committee 2002-2003

President	John G. Hildebrand, PhD	University of Arizona
President-Elect	John W. Scott, PhD	Emory University
Past-President	Stuart J. Firestein, PhD	Columbia University
Secretary	John I. Glendinning, PhD	Barnard College
Treasurer	Debra A. Fadool, PhD	Florida State University
Membership Chair	Mary T. Lucero, PhD	University of Utah
Program Chair	Charles Derby, PhD	Georgia State University
Councilors	M. Scott Herness, PhD	Ohio State University
	Linda B. Buck, PhD	Hutchinson Cancer ResearchCenter

Program Committee 2002-2003

Charles Derby (chair), John Boughter, Pamela Dalton, Valerie Duffy, John Glendinning, Scott Herness Tim McClintock, Paul Moore, Dean Smith, Alan Spector, Suzanne Sollars, Matt Wachowiak

Cover design by Amy Horner, doctoral student, Georgia State University

1 Givaudan-Roure Lecture

MULTICOMPONENT SIGNALS IN ANT COMMUNICATION

Hoelldobler B. *Theodor Boveri-Institut, University of Wuerzburg, Wuerzburg, Germany*

The remarkable ecological success of social insects, and in particular of ants, is based largely on two key features of insect societies: cooperation and communication. Cooperation and division of labor is not possible without communication, and in fact, the communication systems in ants are very diverse. The chemical communication signals are often multicomponent signals that can be produced in single exocrine glands, but they can also be composed with secretions from several glands. Chemical signals can be further combined with stimuli from other sensory modalities such as vibrational or tactile stimuli. These kinds of accessory signals usually serve as modulatory communication, lowering the response threshold in the recipient for the actual releasing stimulus. They can also function as "specifyers", or "functionally referential" signals, when combined with less specific behavioral releasers, such as a trail pheromone that is used in several contexts. Communication is essential for the functioning of the insect society, but it is also an important feature in regulating intraspecific competition among neighboring ant societies. In animal species that live in social groups, contests for limited resources are usually not between single individuals; instead, groups of individuals compete as a unit. In such cases differences in the number of individuals per group determine the outcome of the contest. In certain ant species, the combatants collectively communicate to their opponents information about their size and "resource holding potential".

2 Slide: Animal Behavior & Olfaction

PHEROMONAL MESSAGES BY MALE ASIAN ELEPHANTS: HONEY OR FRONTALIN?

Rasmussen L.E.[1], Riddle H.S.[2], Greenwood D.R.[3] *[1]Biochemistry, Oregon Graduate Institute of Science & Technology, Beaverton, OR; [2]Riddle's Elephant Sanctuary, Greenbrier, AR; [3]Gene Technologies, HortResearch, Auckland, New Zealand*

The state of musth in male Asian elephants (*Elephas maximus*) evidently plays a subtle, but decisive role prior to mating by extensively affecting inter-elephant behavior. During musth males experience internal physiological changes including elevation of serum androgens, triglycerides, ketones and pH, and release exudates reflecting these changes from their breath, urine and temporal gland, a facial gland unique to proboscideans. Sub-adult males during short "moda" musths release sweet-smelling compounds; whereas older, more socially mature males secrete malodorous ketones together with a bicyclic ketal, frontalin. Concurrent behavioral and chemical studies of individual wild and captive elephants demonstrate that moda males are curious about, but avoid: (1) older, secreting musth males, (2) playback samples of collected temporal gland secretion (TGS) from older males, and (3) frontalin samples. Conversely moda males, or their sweet exudates, elicit little response from older males. Female elephants in the wild are indifferent to moda males but responsive (dependent on their hormonal and reproductive status) chemosensorily and behaviorally toward adult males or frontalin samples. In captivity, responsive follicular-phase females often demonstrate mating-related behaviors subsequent to chemosensory responses to frontalin. Postulating that TGS proteins may have a similar perireceptive role to that of elephant albumin (and Z-7-dodecenyl acetate), we present initial studies on frontalin-TGS protein interactions.

3 Slide: Animal Behavior & Olfaction

SEX AND THE SINGLE CELL: MECHANISMS OF SPERM CHEMOATTRACTION IN LAMINAR SHEAR FLOWS

Riffell J.A.[1], Zimmer R.K.[2] *[1]Department of Biology, University of California, Los Angeles, California; [2]Department of Biology, Neurosciences Program and Brain Research Institute, University of California, Los Angeles, California*

Chemical communication between sperm and egg is evident among taxa with highly divergent reproductive strategies. Sperm activation and chemotaxis occur in marine animals that broadcast gametes into the sea, as well as in terrestrial organisms (including humans) with internal fertilization. Chemically mediated behavior is thus a key component of sperm-egg dynamics, whether in the turbulent ocean environment or within a mammalian reproductive tract. Surprisingly, few sperm attractants have been fully identified. Our recent discovery of L-tryptophan as a potent attractant to abalone (*Haliotis rufescens*) sperm offered the rare opportunity to quantify how navigation affects gamete encounter rates and fertilization success. Experiments were performed in laminar-shear flows that simulated important aspects of small-scale turbulence within natural ocean habitats. Swimming behavior and fertilization rates were determined over a wide range of shears using a Taylor-Couette apparatus and a new application of infrared laser and computer-assisted video imaging technologies. Sperm behavioral responses to manipulations of the natural tryptophan gradient around individual eggs revealed that both chemotactic and chemokinetic effects significantly enhanced gamete encounter rates. Moreover, laminar shears dramatically stretched concentration fields of tryptophan around individual eggs, distributing this attractant well beyond broadcast distances otherwise produced by diffusion alone. Thus, even at microscopic scales, physics tightly constrains the chemical signaling process, dictating sperm navigation.

4 Slide: Animal Behavior & Olfaction

OLFACTORY INVESTIGATION OF CONSPECIFIC ODORS IN THE OPOSSUM *MONODELPHIS DOMESTICA* .

Zuri I., Su W., Halpern M. *Anat. & Cell Biol., SUNY Downstate Health Sciences Center, Brooklyn, NY*

Gray short-tailed opossums, South-American marsupials, commonly scent-mark. Both sexes mark their environment with their flanks and heads, but rarely urine-mark. Males also mark with their suprasternal gland. While females have an induced estrus following their exposure to males´ bedding, the role of their body odors in conspecific communication is still unclear. We determined the effect of male and female body odors on the investigatory response of the opposite sex. Each opossum was presented with two cotton balls, one with the test odor and the other with a control. The time spent in snout-cotton ball contact during a one-min period, from the first contact with one of the cottons, was analyzed. Male investigation of urine of prepubertal and of estrus females did not differ significantly compared to water control, but they investigated urine and flank odors of non-estrous females significantly more than controls (Paired t-test, $df = 8$, $P = 0.005$ and $P = 0.001$, respectively). Females did not investigate male urine more than control, but investigation of the male suprasternal gland and mandibles was significantly more vs the controls (Paired t-test, $df = 7$, $P = 0.001$ for both tests). We relate the lack of male urine marking and the lack of female interest in male urine to the semi-arid habitat of the opossums where urine marking may lead to dehydration, and is more volatile than glandular secretions. Males were attracted to odors of non-estrus females that may respond to estrus induction rather than of estrus females that could be already fertilized by the time of approach to them.

5 Slide: Animal Behavior & Olfaction

RABBIT PUPS CAN ORIENT TO THE NEST BY SMELL FROM BIRTH

Hudson R.[1], Garay-Villar E.[2],[4]Maldonado M.[2], Coureaud G.[3] [1]*Biologia Celular y Fisiología, Instituto de Investigaciones Biomedicas UNAM, Mexico;* [2]*Biología Celular y Fisiología, Instituto de Investigaciones Biomedicas UNAM, Mexico;* [3]*Ethology and Sensory Psychobiology, Centre Europeen des Sciences du Gout, Dijon, France*

Rabbits are born into a nest of dried grass and fur. The doe leaves them immediately, only returning to nurse for 3-4 min once a day. Pups are not brooded nor retrieved, and are dependent on the nest for early survival. Since they have a well functioning olfactory system, we tested whether they can orient to the nest by smell. On the day of birth four independent groups of pups were tested individually for 6 min in a side preference arena. In G1 (n=22/12 litters), pups tested after 4-8 h in the nest spent more time on the side immediately above their nest material (mean 81%, SD 21%). In control G2 (n=19/14 litters), pups tested as for G1 but without nest material spent a mean 51% (SD 27%) of time on the side corresponding to the side with nest material in G1. In G3 (n=7/4 litters), pups delivered by oxytocin injection and removed from the nest box as they were born spent a mean 68% (SD 23%) of time on the side above the nest material. In control G4 (n=18/18 litters), pups removed from the nest as for G1, spent a mean 43% (SD 35%) of time on the side above a novel odorant, orange essence. An ANOVA followed by post hoc Fisher's PLSD showed that G1 and G3 tested with nest material spent significantly more time on the stimulus side than either of the control groups ($p<0.05$). Thus, rabbit pups can orient to the nest by smell after little or no postnatal experience. Present work is directed to identifying the stimuli in the nest eliciting this "inborn" preference. Support from PAPIIT IN217100 (RH) and the Fyssen Foundation (GC)

6 Slide: Animal Behavior & Olfaction

PROLONGED EXPOSURE TO SOCIAL ODORS ALTERS SUBSEQUENT SOCIAL INTERACTIONS IN CRAYFISH

Bergman D.A.[1], Moore P.A.[1] [1]*Biological Sciences, Bowling Green State University, Bowling Green, OH*

Aggression is important for the acquisition of resources. Individuals that obtain an elevated social status through agonistic interactions often have increased access to resources. Chemical communication via pheromones in crayfish is used for the recognition of aggressive state by relaying information about social status. This experiment examined the effects of social odors, predominately urine, on the behavior of crayfish that did not have any physical contact with a "sender". Chemical communication in the absence of physical contact may affect the behavior of the "receiver" in a way that the signal reduces or increases the likeliness of a conflict. Crayfish were exposed to one of four different odor types: dominant, subordinate, naïve, or tank water. The odor types were created by giving a crayfish a winner, loser, or no fight experience for five days in a separate fight arena and then placed the crayfish in a tank where the odors were carried to a "receiver" tank. The "receiver" crayfish were exposed to one of the odors for five consecutive days and on the fifth day the odor "receiver" was introduced to a naïve crayfish. The "receiver" that detected an odor from a prolonged "winner" experience behaved as a subordinate crayfish. Conversely, a crayfish exposed to the odor of a prolonged "loser" experienced crayfish became more aggressive. The conclusion drawn was that exposure to status odors alter subsequent interactions of conspecifics in a way that suggests neurochemical changes in the "receiver."

7 Slide: Animal Behavior & Olfaction

MODULATION OF OLFACTORY ACUITY IN MICE: EFFECTS OF STIMULUS CONCENTRATION

Cleland T.A.[1], Narla V.A.[1] [1]*Dept. Neurobiology & Behavior, Cornell University, Ithaca, NY*

Many factors have been shown to influence olfactory perceptual acuity in rats and mice. In rats, the nature of the task significantly influences measurements of olfactory acuity (Cleland *et al.*, 2002), as do cholinergic and other modulatory projections to the olfactory bulb (Linster and Cleland, 2002), supporting the position that olfactory acuity is not a parameter to be maximized, but to be modulated, pursuant to a learned, situation-dependent, and ethologically appropriate breadth of categorization. We here demonstrate, in CD-1 outbred mice, that higher odorant concentrations reduce behavioral generalization between similar odorant stimuli in a rewarded operant task. Specifically, when mice were taught that one member of a homologous odorant series was rewarded, they generalized this contingency to a narrower range of neighboring odorants when the odorants were presented at higher concentrations. Results were replicated for acid, acetate, and aldehyde series presented at 0.01 and 1.0 Pa. As odor concentration can be said to influence conditioned stimulus eligibility (Sutton and Barto, 1990; Pelz *et al.*, 1997), we discuss the relationship between this learning parameter and olfactory perceptual acuity.

8 Slide: Animal Behavior & Olfaction

CONFIGURATIONAL AND NON-CONFIGURATIONAL INTERACTIONS BETWEEN ODORANTS IN BINARY MIXTURES

Linster C., Wiltrout C., Dogra S. *Neurobiology and Behavior, Cornell University, Ithaca, NY*

Studies on odor mixture perception suggest that while odor components can often be identified in mixtures, mixtures can also give rise to novel perceptual qualities which are not present in the components. Using an olfactory habituation task, we evaluate how the perceptual similarity between components in a mixture affects the perceptual quality of the mixture itself. Rats perceived binary mixtures composed of similar components as different from their two components, whereas binary mixtures composed of dissimilar components were perceived as very similar to their components. We then show that for both types of mixtures, pretraining component A reduces subsequent learning about component B when trained in the presence of A. Funded by the Alfred P. Sloan Foundation

9 Symposium: In Sync: Temporal Coding & Encoding Time in the Olfactory System

IN SYNC: TEMPORAL CODING AND ENCODING TIME IN THE OLFACTORY SYSTEM

Christensen T.A.[1], Caprio J.T.[2] [1]*ARL Division of Neurobiology, University of Arizona, Tucson, AZ;* [2]*Zoology and Physiology, Louisiana State University, Baton Rouge, LA*

A half-century of research in diverse organisms shows that even a single odor pulse can evoke a pattern of activity in the brain that is temporally as well as spatially dynamic. In studying odor-evoked responses in the primary olfactory centers of different species, an increasing number of studies reveal complex temporal patterning across olfactory glomeruli, but it remains unclear exactly how network dynamics function in olfactory coding. Network oscillations are ubiquitous in olfactory systems, but it is not clear whether oscillations are functionally relevant. In some studies, oscillatory dynamics modulate the pattern of synchronized firing evoked by odors, whereas in other studies, the timing of odor-evoked neural synchrony is neither oscillatory nor odor-specific, but is modulated by the dynamics of the stimulus itself. The goal of this symposium is to assemble experts who use a range of different experimental systems to study how temporal patterning might be used in the recognition and discrimination of sensory signals. Charles Gray will offer a perspective from the vertebrate visual system which has a long and rich history in dynamic processing. Hartwig Spors will discuss current ideas about olfactory coding based on imaging studies. Alan Gelperin will discuss the latest evidence in mollusks that oscillatory dynamics play a role in odor-information processing and learning. Hong Lei will discuss evidence from the moth which shows that, in contrast to studies in other insects, the pattern of odor-evoked spike synchrony in olfactory glomeruli is not phase-locked to field oscillations in either the antennal lobe or the mushroom body. A comprehensive question-answer session will follow.

10 Symposium: In Sync: Temporal Coding & Encoding Time in the Olfactory System

SYNCHRONOUS ACTIVITY IN THE VISUAL SYSTEM: HISTORY AND CURRENT STATUS

Gray C.[1] [1]*Montana State University, Bozeman, MT*

Synchronous activity is currently an intensely studied subject in neurophysiology. In vision, where it remains controversial, it has been attributed to a variety of functions ranging from scene segmentation to visual awareness. Prior to 1980, synchronous activity wasn't even on the radar screen of most visual neuroscientists. It was relegated to arcane subjects such as olfaction, and synaptic plasticity. Sensory processing was commonly studied by mapping receptive fields and cortical areas. This began to change, however, with the gradual realization that visual perception is fundamentally a distributed, combinatorial process. A theory was needed to incorporate distributed processing, combinatorial flexibility and temporal dynamics. This theory, preceded by concepts introduced by Hebb and others, emerged in the form of two largely ignored papers by Milner (1974) and Malsburg (1981). They proposed that perceptual grouping is mediated dynamically by distributed assemblies of synchronously firing neurons. Later, this model captured widespread attention with the discovery of stimulus dependent synchronous firing in visual cortex. Subsequent work has revealed that synchronous activity is a robust and general feature of neuronal networks. We have a much better understanding of how it is generated, and there are many interesting correlates with perceptual, motor and cognitive functions. The jury is still out, however, regarding the functions of synchronous activity, and critics abound. I will briefly review the history of synchronous activity, discuss the pros and cons of the correlation model, and layout some arguments for why I think synchronous activity is fundamentally important for many brain processes.

11 Symposium: In Sync: Temporal Coding & Encoding Time in the Olfactory System

LEARNING ABOUT ODORS WITH OSCILLATIONS AND WAVES

Gelperin A.[1] [1]*Monell Chemical Senses Center, Philadelphia, Pennsylvania*

The olfactory system performs impressive feats of pattern recognition and pattern matching on the spatial and temporal patterns of sensory input elicited by odor stimulation. The role of oscillatory dynamics in the computation of odor identity and concentration is a subject of great interest in a variety of model systems. We have pursued this issue in a molluscan model system (Limax) whose olfactory system displays many of the design features of the mammalian olfactory system (J Exp Biol 202:1855, 1999). The Limax olfactory bulb analog (OBA) has oscillatory dynamics of its local field potential and propagates activity waves from apex to base. The oscillatory dynamics arises from a network of coupled bursting local inhibitory neurons that have a gradient of excitability along the apical-basal axis. Odor memories appear to be stored in bands of olfactory neurons in the OBA, which our model attributes to the synchronizing effect of local shared oscillations among odor-driven interneurons. Recent 2-photon imaging of calcium dynamics in neurites of the local inhibitory interneurons clarifies how activity propagates in the network of coupled bursting neurons. The dependence of oscillatory dynamics and odor memory storage on endogenous nitric oxide generation in the OBA is a general feature of mammalian and molluscan olfactory systems. Our model makes testable predictions about how oscillatory dynamics guides odor memory formation in the molluscan OBA, which can be tested in the Limax OBA and in the mouse olfactory bulb. Supported by NIH grant MH56090.

12 Symposium: In Sync: Temporal Coding & Encoding Time in the Olfactory System

SPATIO-TEMPORAL DYNAMICS OF RECEPTOR NEURON INPUT TO THE MAMMALIAN OLFACTORY BULB

Spors H.[1], Wachowiak M.[2], Cohen L.B.[2], Friedrich R.W.[1] [1]*Max-Planck-Institute for Medical Research, Heidelberg, Germany;* [2]*Physiology, Yale University, New Haven, Connecticut*

Odors evoke dynamic glomerular activity patterns in the olfactory bulb (OB, Spors and Grinvald, 2002). To analyze the dynamics of these patterns at the level of input to the OB, we selectively loaded olfactory receptor neurons with Calcium Green dextran and imaged afferent glomerular calcium dynamics in freely breathing or artificially sniffing, anesthetized mice (Wachowiak and Cohen, 2001). Glomerular odor responses differed in response latency, rise time, decay time, and modulation by sniffing. In response to esters and hydrocarbons, caudo-lateral glomeruli generally exhibited faster responses and more pronounced respiratory modulation. However, neighboring glomeruli could also exhibit different temporal response characteristics. Temporal response characteristics of individual glomeruli depended on glomerulus identity, odor identity, odor concentration, sniffing frequency, and flow rate. Changing from freely breathing to artificially sniffing altered the degree of respiratory response modulation, while differences in response latency, rise time, and amplitude across glomeruli and odors were preserved. The temporal response properties were consistent for equivalent glomeruli in different preparations. Hence, the spatial pattern of glomerular input can change significantly over time in a stimulus-specific manner. The spatio-temporal dynamics of afferent activity patterns therefore need to be considered in models of olfactory coding. Support by BMBF, MPG, NIH DC00378, DC04938, and DC05259.

13 Symposium: In Sync: Temporal Coding & Encoding Time in the Olfactory System

CENTRAL ENCODING OF OLFACTORY INFORMATION THROUGH TRANSIENT, NON-OSCILLATORY SYNCHRONIZATION OF NEURAL ENSEMBLES

Lei H.[1], Christensen T.A.[1], Hildebrand J.G.[1] *[1]ARL Division of Neurobiology, University of Arizona, Tucson, AZ*

The distributed patterns of neuronal firing evoked by odors are both spatially and temporally complex, making it difficult to decipher the rules by which olfactory networks identify and discriminate odours. In the primary olfactory center in insects, the antennal lobe (AL), some studies have shown that coherent firing among AL projection neurons (PNs) may be phase-locked to odor-evoked oscillations, but it remains unclear whether oscillatory synchronization is necessary for odor recognition and discrimination. How, for example, does the brain use this time-delimited mechanism to encode a sensory signal that is itself temporally unpredictable? We find that in the olfactory system of the moth, odors evoke 10-50 Hz oscillations in the AL and the mushroom body (MB; a higher-order network that receives input from the AL), but the oscillations within or between these two processing stages are not temporally coherent. Moreover, in response to an odor pulse, the timing of PN firing is not phase-locked to oscillations in either the AL or MB. The majority of PN spikes occur during the non-oscillatory phase of the LFP recorded in the same glomerulus, and the correlation between PN synchrony and LFP oscillations remains low before, during, and after odor stimulation. These new results demonstrate that local network rhythmicity is not functionally related to the odor representations of these AL output neurons. We provide evidence that while oscillatory synchronization of PNs can occur in the olfactory system under certain stimulus conditions, it does not serve an obligatory function in the central representation of odors. Supported by NIH grant R01-DC-02751 to JGH.

14 Poster: Olfactory Neurogenesis & Cell Death

MATRIX METALLOPROTEINASE EXPRESSION IN THE OLFACTORY EPITHELIUM

Tsukatani T.[1], Fillmore H.[2], Hamilton H.[2], Holbrook E.[3], Costanzo R.M.[3] *[1]Otorhinolaryngology, Kanazawa University, Kanazawa, Japan; [2]Surgery, Virginia Commonwealth University, Richmond, Virginia; [3]Physiology, Virginia Commonwealth University, Richmond, Virginia*

Matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs), have been implicated in many biological processes including cell turnover, development, outgrowth, homeostatic and metastatic processes. We used western blot analysis and immunohistochemistry to determine if MMP-2 and its activity related proteins TIMP-2 and membrane type 1 matrix metalloproteinase (MT1-MMP) are present in the olfactory epithelium of mice and if activity levels change during recovery from unilateral olfactory bulb ablation (bulbectomy). Olfactory bulbectomy is a well established model for the study of neural degeneration, regeneration and axon outgrowth. Our results are the first to report the expression of MMP-2 among basal and immature cells in the olfactory epithelium. We found that MMP-2 and MT1-MMP levels decreased immediately after bulbectomy, and reach a low point when TIMP-2 levels were at their peak. At this time point in recovery the degenerated mature olfactory neurons were replaced by newly differentiated immature neurons. These findings suggest that MMPs and TIMPs may play a role in neurogenesis, maturation and axon outgrowth of replacement neurons in the olfactory system. Supported by NIH DC00165

15 Poster: Olfactory Neurogenesis & Cell Death

BIOCHEMICAL CHARACTERIZATION OF CELL SURFACE ANTIGEN OF GLOBOSE BASAL CELLS

Jang W.[1], Schwob J.E.[1] *[1]Department of Anatomy and Cellular Biology, Tufts University, Boston, MA*

Globose basal cells (GBCs) are crucial for the capacity of the OE to recover after injury and to maintain neurogenesis. Our understanding of the GBC population would be improved by characterizing their biochemical phenotype and looking to correlate that with functional capacity. To that end we have been studying the antigen recognized by MAb GBC-3, which marks a population of GBCs. GBC-3 immunoreactivity is markedly increased when the OE is rapidly reconstituting after injury, e.g., the first week after MeBr exposure. The antigen is cell-surface, as it stains living cells, and attached to the membrane by a GPI linkage, as it is released by PLC digestion. We took advantage of its expression by the GBC-derived NIC cell line for purposes of biochemical characterization. The antigen expressed by acutely isolated GBCs and by NIC cells is a glycoprotein of 40 kDa MW. Blots of 2-D gels show a range of pIs, suggesting a variety of posttranslational modifications. To elucidate how this antigen is regulated during cell differentiation, NIC cells were driven toward either a GBC-like vs. a neuronal state using the growth factors FGF-2 and TGF-βs, respectively. The expression level of the GBC-3 antigen is attenuated in response to TGF-βs, as NIC cells are becoming more neuronal-like. The cell line data is consistent with our in vivo results, since GBC-3 antigen is expressed at peak levels during the massive proliferation of GBCs after injury. Thus, GBC-3 has the potential to be a useful tool for studying the behavior of GBCs during epitheliopoiesis, and of neuronal stem cells, in general. Supported by R01 DC02167.

16 Poster: Olfactory Neurogenesis & Cell Death

QUIESCENT GLOBOSE BASAL CELLS ARE PRESENT IN THE OLFACTORY EPITHELIUM

Chen X.[1], Schwob J.E.[1] *[1]Department of Anatomy & Cellular Biology, Tufts University School of Medicine, Boston, MA*

The olfactory epithelium (OE) maintains the capacity for neurogenesis and reconstitutes both neuronal and non-neuronal cells following direct experimental injury. However, the nature of the multipotent progenitor cell in the OE that gives rise to all cell types remains uncertain. We here seek to discover whether the OE harbors a population of quiescent globose basal cells (GBCs) because quiescence and its activation by lesion is a hallmark of stem cells and of multipotent progenitors. Quiescent GBCs were identified in two ways: (1) BrdU was injected subcutaneously to 3 d old rats daily for 3 days. Cells retaining the label for 4 weeks and not labeled by horizontal cell (HBC) marker (Cytokeratin 5/6) and neuronal marker (Tuj-1) were identified quiescent GBCs. (2) Antibody against p27KIP1, a cyclinD/cdk4 binding protein expressed by cells in G0, along with HBC and neuronal markers, was used to visualize quiescent GBCs in normal, post MeBr-lesion, and post bulbectomized rat OE. Our results showed a small population of GBCs are labeling-retaining cells (LRCs), which is a characteristic for slow-cycling stem cells. Quiescent GBCs exist in normal OE and are maintained after bulbectomy. After MeBr lesion, the quiescent GBC population disappeared, indicating they were activated and contributed to the OE reconstitution. Collectively, our data suggest that quiescent GBCs are present in the normal OE, and they participate in the regeneration of all cell types following MeBr lesion. Supported by DC 02167.

17 Poster: Olfactory Neurogenesis & Cell Death

HEME OXYGENASE-1 AND HEME OXYGENASE-2 HAVE DIFFERENT ROLES IN THE REGENERATION OF OLFACTORY RECEPTOR NEURONS AFTER DETERGENT ABLATION

Tu Y.[1], Chen J.[1], Moon C.[1], Ronnett G.V.[2] [1]*Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD;* [2]*Neuroscience and Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD*

Heme oxygenase (HO) is implicated in protection against oxidative stress, proliferation, and apoptosis in many cell types, including neurons. In this study, we utilized detergent ablation of olfactory epithelium containing olfactory receptor neurons (ORNs) as a model to investigate the roles of HO-1 and HO-2 in neuronal proliferation and survival. Ablation was performed by nasal irrigation with aqueous solutions of Triton X-100. Olfactory tissues were harvested at two weeks after ablation and immunohistochemistry was performed to check the expression of different markers. Two weeks after ablation, increased neurogenesis in the epithelium produced a prominent increase in immature neurons. Mature neurons had also re-appeared. Immature neurons were reduced in HO-1 and HO-2 null mice and mature neurons were reduced in HO-2 null mice. HO-1 and HO-2 null mice displayed decreased proliferation of neuronal precursors. Apoptosis was increased in HO-2 null mice. Cyclic GMP immunostaining was reduced in ORNs in both genotypes, providing direct evidence that HO mediates cGMP production. Bilirubin immunostaining was reduced in HO-2 null mice. These results suggest that HO-1 and HO-2 have different functions in neuronal regeneration: both are involved with proliferation, but only HO-2 influence survival. The results also support a role for the CO-cGMP signaling system and bilirubin in regeneration of ORNs. Supported by NIH grants from NIDCD DC-02979 and NINDS NS-39657 to GVR.

18 Poster: Olfactory Neurogenesis & Cell Death

NITRIC OXIDE INDUCES CELL PROLIFERATION DURING THE NEUROGENESIS OF RAT OLFACTORY EPITHELIUM *IN VITRO*

Sülz L.[1], Mackay-Sim A.[2], Ferón F.[2], Iturriaga R.[1], Bacigalupo J.[3] [1]*Facultad de Ciencias Biológicas, Universidad Católica de Chile, Santiago, Chile;* [2]*Griffith University, Brisbane, Queensland, Australia;* [3]*Millenium Institute CBB, Facultad de Ciencias, Universidad de Chile, Santiago, Chile*

The sensory neurons of the olfactory epithelium are continually renewed during the entire lifespan in mammals. The stem cells responsible for cellular replacement are found among the basal cells of this epithelium. The enzyme nitric oxide synthase (NOS), which generates the diffusible second messenger nitric oxide (NO), is transiently expressed during neurogenesis in the olfactory bulb and the olfactory epithelium of adult animals. We examined the possibility that NO has a role in neurogenesis using primary cultures of basal cells from olfactory epithelia of adult rats, containing exclusively non-neuronal cells. Treatment of the cultures with EGF/FGF-2 and TGF-&beta2 stimulated cell proliferation and differentiation to immature neurons (tubulin &betaIII and neurofilament-200 expression), respectively. We detected the expression of the neuronal (nNOS) and inducible (iNOS) enzyme isoforms, but not the endothelial isoform (eNOS), under both treatments. Furthermore, nNOS is co-expressed with tubulin &betaIII (immature neurons) but not with cytokeratin (flat basal cells and supporting cells). TRIM, an inhibitor of nNOS and iNOS, reduced proliferation and increased differentiation in a dose-dependent manner. These results support the notion that NO participates in neurogenesis, stimulating cell proliferation in the olfactory epithelium. CONICYT Doctoral Fellowship (LS) and Mideplan ICM P99-031-F (JB).

19 Poster: Olfactory Neurogenesis & Cell Death

CELL PROLIFERATION AND GROWTH IN *MANDUCA SEXTA* ANTENNAL IMAGINAL DISCS

Fernandez K.A.[1], Kobres R.P.[1], Vogt R.G.[1] [1]*Biological Sciences, University of South Carolina, Columbia, SC*

The antenna of the adult tobacco hornworm, *Manduca sexta* (*M. sexta*), detects and processes odorants. It develops at the larval stage with the growth and differentiation of the antennal imaginal disc. The final instar larva consists of an eight day period divided into a prewandering (4 days) and wandering stage (4 days) occurring before pupation. Although developmental aspects of the adult antenna have been studied, questions remain regarding how the imaginal disc develops from a flattened sac composed of two cell layers (i.e. the peripodial membrane and columnar epithelium) to a specialized and segmented structure. Our interest is to determine whether cell proliferation events in the final instar larva antennal imaginal disc contribute to the formation of the adult structure. Fluorescent antibodies to a cell cycle marker, phosphorylated histone H3, and a nuclear counterstain, DAPI, were used to visualize patterns of cell division using confocal microscopy. Our findings show proliferative events occurring mostly on the tissue facing the lumen between the peripodial layer and the folded columnar epithelium. At the same time, there is more division occurring towards the bulbous or proximal end of the disc. By wandering day four (day 8), cell proliferation can no longer be detected, using H3, suggesting that cell proliferation in the antennal imaginal disc is temporally regulated. These results indicate the need to study more aspects of the cellular and molecular events taking place in the antennal imaginal disc.

20 Poster: Olfactory Neurogenesis & Cell Death

FUNCTIONAL ASPECTS OF NEUROGENESIS IN THE ADULT OLFACTORY BULB

Belluzzi O.[1], Benedusi M.[1], Ackman J.[2], Loturco J.J.[2] [1]*Dept. Biology - Sect. Physiology & Biophysics, Universita' di Ferrara, Ferrara, Italy;* [2]*Dept. Physiology and Neurobiology, University of Connecticut, Storrs, CT, U.S.A.*

New cells expressing neuronal markers are generated postnatally at the subventricular zone of the forebrain, and from there they migrate into the olfactory bulb (OB) following a well defined pathway, the rostral migratory stream (RMS). However it is still unclear whether newly generated cells (NGC) that express neuronal markers become functional neurones. To fill this gap we combined a retroviral mediated cell lineage and patch-clamp electrophysiology. We have identified one-month old neurones with functional properties similar to those of mature periglomerular (PG) and granule cells in the adult OB. From these cells we recorded action potentials in response to the injection of depolarising currents, and we have isolated and characterised Na+ and K+ currents. About 50% of the NGC were PG cells; the remaining ended their migration between internal plexiform and mitral cell layers, or in the granule cell layer. The migrating neuroblasts within the RMS show only a small delayed rectifier K-current, to which an A-current is added when the OB is reached. The mature cells lose the delayed rectifier K+-current, and only display A- and Na+ currents. Finally, by recordings action potentials and excitatory synaptic currents in response to stimulation of the olfactory nerve. These results show that endogenous neuronal progenitors produce new neurons that integrate into previously established and functioning circuits. Supported by grants from Fondazione Caricento (OB) and NIH (MH56524, J.J.L.)

21 Poster: Olfactory Neurogenesis & Cell Death

NEUROGENESIS IN THE CENTRAL OLFACTORY PATHWAY OF ADULT DECAPOD CRUSTACEANS: IDENTIFICATION OF NEUROBLASTS

Schmidt M.[1] [1]*Georgia State University, Atlanta, GA*

In adult decapod crustaceans, pulse chase experiments with BrdU (5-bromo-2'-deoxyuridine) combined with immunostainings for neuropeptides have demonstrated continuous neurogenesis in the neuronal soma clusters of the central olfactory pathway (Schmidt, M. Brain Res. 762:131-143,1997; Schmidt, M. J. Neurobiol. 48:181-203, 2001). Proliferation in these soma clusters occurs in small proliferative zones at the border between soma clusters and neuropil. About 1 month after a single BrdU-injection, all BrdU-positive cells have undergone one cell division and have left the proliferative zones. This leaves the question how new proliferating cells are generated, specifically if self-renewing neuroblasts are present. To answer this question multiple BrdU-injections (7 injections with 6 h time intervals) were performed in adult spiny lobsters, *Panulirus argus*. These multiple BrdU-injections - in contrast to single injections – frequently resulted in the labeling of one or few additional nuclei located outside of but close to the proliferative zones. These additional nuclei differ from those in the proliferative zones by a more spherical shape, a larger size, and a looser chromatin. Furthermore they are surrounded by a very compact and distinctive sheath of glial cells. From these observations I conclude that the additionally labeled cells might represent the neuroblasts that drive adult neurogenesis in the central olfactory pathway of decapod crustaceans. The glial sheath might be involved in maintaining the life-long self-renewal capacity of these adult neuroblasts. Supported by Deutsche Forschungsgemeinschaft (Schm 738/8-2).

22 Poster: Olfactory Neurogenesis & Cell Death

CIRCADIAN RHYTHMICITY TO NEUROGENESIS IN THE OLFACTORY ORGAN AND BRAIN OF SPINY LOBSTERS

Horner A.J.[1], Nduku V.[1], Vu V.[1], Harrison P.J.[1], Derby C.D.[1] [1]*Biology, Georgia State University, Atlanta, GA*

Continuous neurogenesis has been described in the olfactory pathway of a variety of organisms, but circadian changes in cell proliferation have only rarely been described in nervous systems. We are aware of just one study (Goergen *et al.* 2002, *J. Neurobiol.* **53**:90-95) describing circadian rhythmicity to neurogenesis of olfactory neurons. We used BrdU to examine circadian changes in neurogenesis in the olfactory organ (antennule) and brain of the spiny lobster, *Panulirus argus*, which is known to have post-embryonic neurogenesis influenced by environmental and physiological factors. In the brains of freshly caught animals held outdoors under natural lighting, we found significant differences in proliferation rates of olfactory interneurons over the course of a day, with peak levels occurring during daylight hours and lower levels at night. Their olfactory organs also showed significant differences in proliferation rates, but the maximum rates of proliferation in the periphery were offset from those in the brain by ca. 12 hr. A qualitatively similar but statistically non-significant pattern of neurogenesis was observed in the brains and antennules of lobsters that had been maintained in the laboratory for extended periods of time. The difference between field and lab results is likely due to the lighting in the lab being dimmer, of limited spectrum, and not under a strict circadian cycle. Possible reasons for the difference in timing of peak proliferation levels between the central and peripheral nervous system are discussed. Supported by NSF IBN-0077474 and NIH DC00312.

23 Poster: Olfactory Neurogenesis & Cell Death

PACAP INHIBITS ORN APOPTOSIS AND REDUCES TRANSIENT K CURRENT THROUGH DIFFERENT INTRACELLULAR PATHWAYS.

Han P.[1], Hegg C.C.[1], Lucero M.T.[1] [1]*Physiology, University of Utah, Salt Lake City, Utah*

We previously showed that pituitary adenylate cyclase activating polypeptide (PACAP) prevents apoptosis in adult mammalian olfactory receptor neurons (ORNs) (Chem. Senses 2002, 27:A97). The inhibition of apoptosis was tightly correlated with reduction of transient K currents (IK_T). Here, we explored the pathways mediating these PACAP effects. ORNs were dissociated from adult Swiss Webster mice and maintained for 24 hrs prior to patch-clamp recording and CaspACE-FITC-VAD-FMK labeling. Four cultures were used per group. The IK_T was converted to current-density by dividing the peak current at +60 mV by the membrane capacitance. Apoptosis was determined by double-blind counting of cells containing activated caspases. We found that (1) PACAP (40 nM) significantly reduced IK_T density from 522 ± 51 pA/pF (0 nM) to 206 ± 52 pA/pF ($p<0.05$). In the presence of PACAP, PLC antagonist U73122 (10 μM) restored the IK_T density to control levels (454 ± 38 pA/pF), while inactive U73343 was similar to PACAP alone with an IK_T density of 191 ± 23 pA/pF. In contrast, SQ22536 (500 μM), an adenylyl cyclase antagonist, did not block PACAP's reduction of IK_T density (222 ± 28 pA/pF). (2) PACAP (40 nM) reduced the % caspace$^+$ ORNs from 66 ± 2% (0 nM) to 55 ± 2%. In the presence of PACAP, neither U73122 (68 ± 3 %) nor SQ22536 (70 ± 4%) reduced the % of caspace$^+$ ORNs. Taken together, these results suggest that reduction of IK_T by PACAP requires activation of the PLC but not the cAMP pathway. However, the inhibition of ORN apoptosis by PACAP involves both PLC and cAMP pathways. This work was funded by NIH NIDCD DC02994 to MTL.

24 Poster: Olfactory Neurogenesis & Cell Death

APOPTOSIS IN THE VOMERONASAL SENSORY EPITHELIUM IN ADULT RATS

Jia C.[1], Halpern M.[1] [1]*Anatomy & Cell Biology, State University of New York Health Science Center at Brooklyn, Brooklyn, NY*

Receptor neurons in the vomeronasal sensory epithelium, like olfactory neurons, undergo continuous turnover. Neurons are generated by progenitor cells in the basal region of the sensory epithelium. Neurogenesis is not homogeneously distributed in the sensory epithelium, with more neurons generated in the regions close to the sensory-nonsensory epithelial junction. The purpose of this study was to determine where the cell death occurs in the vomeronasal sensory epithelium. Using TUNEL method to detect apoptosis, we found that apoptotic figures were distributed in both basal and receptor cell layers of the sensory epithelium. More apoptotic figures were found in the regions close to the sensory-nonsensory epithelial junction, where more proliferating cells were found using BrdU labeling method. We are currently using combined TUNEL and BrdU labeling to analyze the temporal and spatial relationship between neurogenesis and apoptosis in the vomeronasal sensory epithelium. Supported by NIH DC 02745, M.H.

25 **Poster: Olfactory Neurogenesis & Cell Death**

INHIBITION OF ORN APOPTOSIS IN THE BAX KNOCKOUT MOUSE

Robinson A.M.[1], Conley D.B.[1], Kern R.C.[1] *[1]Otolaryngology-HNS, Northwestern University, Chicago, IL*

Bax is a central apoptotic protein in many cell types, mediating an increase in mitochondrial membrane permeability in response to pro-apoptotic signals. Cytochrome c then leaks into the cytoplasm from the mitochondria activating proteases such as caspase-3, which mediate cell death. In the olfactory system removal of the olfactory bulb triggers a wave of olfactory receptor neuron (ORN) apoptosis. Earlier studies in our laboratory demonstrated that bulbectomy is associated with a rise in bax and pro-caspase-3 mRNA. The current study utilized bax knockout mice to further assess the role of this protein in ORN apoptosis. Wild-type and bax KO mice underwent unilateral bulbectomy and were analyzed at 1, 2, 3, 4, 5 and 9 days post-surgery. Immunohistochemistry was performed on paraffin-embedded head sections to detect the active form of caspase-3. High levels of activated caspase-3 protein were detected at all time points on the bulbectomy side in the Wild-type mouse. This was associated with a marked decline in OMP (Olfactory Marker Protein) staining, shrinkage of the olfactory epithelium (OE) and a 75% decrease in ORN number when compared to the un-operated side. In the bax KO mouse, active Caspase-3 was present on the operated side, but substantially reduced when compared to the Wild-type mouse. The reduction in OMP staining, OE shrinkage and ORN loss in the bax KO were dramatically less than that seen in the Wild-type mouse. Effects were most apparent at 9 days post injury, indicating that apoptosis was inhibited, not just delayed. These results provide further evidence that Bax plays a central role in ORN apoptosis, at least following trauma, suggesting the potential for therapeutic intervention in humans.

26 **Poster: Olfactory Neurogenesis & Cell Death**

CELLULAR SENESCENCE IN HUMAN OLFACTORY CULTURES

Peoples R.[1], Patel K.[1], Gomez G.[2], Rawson N.[1] *[1]Monell Chemical Senses Center, Philadelphia, PA; [2]University of Scranton, Scranton, PA*

Human olfactory cultures (HOC) established from nasal biopsies have been used as an in vitro model to study odor elicited calcium changes (Gomez et al., 2000). Observations of these cultures over time suggested that senescence occurs with increasing passage number and that the rate of aging varies among cultures derived from different biopsies. To characterize this senescence process we used morphometric methods to study growth rates of cultures derived from six healthy subjects aged 32 – 51yrs. As the HOC age, there is a population shift and a morphological change in the cell populations. The younger HOC have elongated, slender cells and phase- bright neurons. With increasing passages, there is a population shift to large, flat cells. Cell counts revealed that while the older cultures appeared to be confluent, this was due to an increase in cell size rather than cell number. In contrast, there was a large increase in the total number of cells across early passages. Also, the number of phase bright neurons decreases as the culture ages and change morphologically, enlarging and becoming granulated. The time for each individual HOC to age varied from passage 8 to passage 14. This may relate to subject characteristics or to the size or composition of the initial biopsy. Cryopreservation at early passages did not substantially alter the rates of growth and senescence. These data provide a foundation for future studies of cellular growth and senescence in the human olfactory epithelium and establish guidelines to maximize culture consistency for physiological studies. Funded in part by DC00214.

27 **Poster: Development of Gustatory Systems**

PERSISTENT SIGNALS FROM THE NOTOCHORD ENHANCE EMBRYONIC TASTE BUD DEVELOPMENT.

Barlow L.A.[1], Calabrese D.W.[1] *[1]Dept. of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO*

During gastrulation, the fate of pharyngeal endoderm (PE) is specified by the notochord. Once gastrulation finishes, the PE has received sufficient notochordal signals to produce taste buds autonomously (Barlow, 2001 Development 128:4573). Because the intimate contact of notochord with PE during gastrulation continues throughout embryogenesis, and because the notochord influences development of other gut regions after gastrulation, we asked if prolonged exposure to the notochord also affected taste bud development in PE. Approaching this question experimentally, we removed specified PE and notochord from mid-neurula stage axolotl embryos, and either cultured PE alone, or allowed PE to form fused explants with notochord. After 10 days in vitro, explants were processed for indirect immunofluorescence to detect differentiated taste buds. Eight +/- 0.7 taste buds formed per PE explant, while fused explants had an average of 15 +/- 0.0 taste buds. Taste buds in fused explants also contained more cells (2.6 +/- 0.6) than those in PE explants (1.95 +/- 0.18). However, our sample size for fused explants (n=2) is currently too small to determine if these numbers differ significantly from control PE (n=12). Nonetheless, later contact with notochord appears to augment development of taste buds in specified PE, causing more and larger taste buds to form. We are now exploring the temporal and spatial limits of this interaction, and assessing which secreted proteins expressed by the notochord enhance taste bud genesis. Supported by NIDCD, DC03947 and DC00244

28 **Poster: Development of Gustatory Systems**

THE ROLE OF EPITHELIAL-MESENCHYMAL INTERACTIONS IN THE DEVELOPMENT OF MURINE TASTE PAPILLAE

Yuskaitis C.J.[1], Barlow L.A.[1] *[1]Dept. of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO*

The early development of the gustatory papillae of mammals occurs independently of contact with ingrowing nerve fibers. Papillary morphogenesis, as well as the expression of BDNF, BMP4 and SHH, all occur in the papillary epithelium of cultured tongue explants. However, the degree to which this patterning of the lingual epithelium requires interaction with the underlying mesenchyme has not been explored. To attack this question experimentally, we have devised a new technique where we culture the epithelium and mesenchyme either separately, or in combination, and examine the expression of papillary marker genes. Lingual explants are removed from E11.5-12 mouse embryos, and cultured for 3 days using a floating filter method (modified from LaMantia et al. 2000 Neuron 28:411). Both BDNF-lacZ and BMP4-lacZ are expressed in the epithelium of gustatory papillae in intact explants, whereas epithelium raised alone fails to express these markers. Our initial impression was that isolated epithelium was undergoing cell death in the absence of mesenchyme. However, staining with Hoechst 33248 revealed these explants possess healthy nuclei within an intact epithelium, and had simply failed to turn on papillary markers. We are now exploring the tissue level interactions necessary for proper gene expression in the epithelium by performing recombination experiments of lingual epithelium with either homotopic or heterotopic mesenchyme. Supported by NIDCD, DC03947 and DC00244

29 Poster: Development of Gustatory Systems

ALTERATION OF FUNGIFORM PAPILLA PATTERN INDUCED BY BRIEF DISRUPTION OF SHH SIGNALING

Liu H.X.[1], Maccallum D.K.[2], Mistretta C.[1] [1]*School of Dentistry, University of Michigan, Ann Arbor, MI;* [2]*Medical School, University of Michigan, Ann Arbor, MI*

We previously used a steroidal alkaloid, cyclopamine (CYCL), to specifically disrupt sonic hedgehog (Shh) signaling in cultures of embryonic rat tongue, and demonstrated roles for Shh in fungiform papilla development and patterning. Fungiform papillae formed in increased numbers and atypical posterior locations on tongues cultured with CYCL for 2 days, compared to controls. To further understand regulation of papilla development, we observed the effects of short CYCL exposure on the pattern of fungiform papillae in whole tongue cultures started at gestational day 14. Tongues were dissected and cultured in medium with 5 uM CYCL for 4, 8, or 12 hr, followed by fresh standard medium with no CYCL for up to 48 hr. Cultures with medium alone or with added CYCL for the entire 48 hr culture period were taken as negative and positive controls. Cultured tongues were analyzed by scanning electron microscopy. Only 4 hr of CYCL exposure was sufficient to cause formation of more prominent fungiform papillae on the posterior oral tongue and an increased number of fungiform papillae on anterior tongue. This effect became more significant with 8 hr of CYCL exposure, and the effect of 12 hr of CYCL exposure was as dramatic as that of 48 hr. Results demonstrate that a brief perturbation of Shh signaling leads to irreversible changes in papilla patterning. Thus, even a short disruption in Shh signaling initiates new molecular programs in tongue epithelium that would not usually support papillae. Supported by NIH NIDCD Grant DC00456 to CM.

30 Poster: Development of Gustatory Systems

EXPRESSION OF BDNF, NGF AND PGP 9.5 IN TASTE BUDS FOLLOWING GLOSSOPHARYNGEAL NERVE SECTION IN MICE

Yee C.L.[1], Finger T.E.[1] [1]*Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO*

BDNF is the neurotrophin that supports gustatory innervation throughout development of the taste periphery. In adult mice, BDNF is expressed by mature taste cells that form synaptic contacts with nerve fibers. Although NGF reportedly does not appear to serve as a gustatory neurotrophin during development, a subset of taste cells in adult mice nonetheless express NGF. To determine whether the expression of these neurotrophins in taste cells is related to gustatory innervation, we investigated the expression of BDNF, NGF and PGP 9.5 at various times following unilateral transection of the glossopharyngeal nerve. During the period of denervation (1-3 weeks) following nerve section, no neurotrophin-expressing cells are present in the denervated taste epithelium. By three weeks after nerve transection, BDNF-immunoreactivity (ir), NGF-ir and PGP 9.5-ir are evident in regenerating nerve fibers. In addition, BDNF-ir, NGF-ir and PGP 9.5-ir taste cells are present in regenerating taste buds. We have measured the diameter of taste buds, counted taste buds and determined the average number of BDNF and NGF immunopositive taste cells per taste bud on the nerve transected vs. unoperated side of the tongue. Results indicate that while there is severe taste bud loss and subtle morphological changes on the nerve-transected side of the tongue; BDNF, NGF and PGP 9.5 are expressed relatively normally in regenerated taste buds. Supported by NIH grant DC05106 to C.L.Y. and DC00244 to T.E.F.

31 Poster: Development of Gustatory Systems

EPITHELIAL OVEREXPRESSION OF BDNF AND NT4 EACH PRODUCES DISTINCT SPATIAL PATTERNS OF ALTERED CHORDA TYMPANI INNERVATION.

Lopez G.F.[1], Krimm R.F.[1] [1]*Anatomical Sciences, University of Louisville School of Medicine, Louisville, KY*

The neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT4) may be important for gustatory nerve fiber targeting as well as neuron survival. Previous results demonstrate that altering the pattern of BDNF or NT4 expression disrupts targeting of the chorda tympani nerve. Specifically, when BDNF (BDNF-OE) or NT4 (NT4-OE) is overexpressed in the basal epithelium of transgenic mice, chorda tympani fibers appear to be misdirected to inappropriate non-taste bud targets. To determine whether BDNF-OE results in the same pattern of altered innervation as NT4-OE, we examined the overall spatial distribution of chorda tympani fibers in BDNF-OE, NT4-OE, and control mice in whole tongues. In NT4-OE mice, chorda tympani nerve branching is reduced and the lateral to medial distribution of nerve fibers is disrupted. In BDNF-OE mice, many fine chorda tympani nerve branches were observed near the epithelial surface; however, few invade fungiform papillae. Innervated fungiform papillae were present only around the intermolar eminence and the ventral tongue tip in NT4-OE mice, while some fungiform papillae on the dorsal tongue tip were innervated in BDNF-OE mice. Therefore, while overexpression of either BDNF or NT4 misdirects chorda tympani fibers, the resulting patterns of altered chorda tympani innervation are different between these two neurotrophins. We plan to determine when chorda tympani innervation patterns are first altered in neurotrophin overexpressing mice. This research is supported by NIH grants DC04763 and DC05252.

32 Poster: Development of Gustatory Systems

EFFECTS OF SODIUM CHANNEL BLOCKERS ON NEUROTROPHIN - INDUCED NEURITE OUTGROWTH IN EMBRYONIC TRIGEMINAL GANGLION

Bai L.[1], Maccallum D.[2], Mistretta C.[1] [1]*School of Dentistry, University of Michigan, Ann Arbor, MI;* [2]*Medical School, University of Michigan, Ann Arbor, MI*

Sensory input and electrical activity can increase synthesis and release of neurotrophins in various brain regions. In turn, neurotrophins such as brain-derived growth factor (BDNF) can open sodium channels and alter electrical activity, generation of action potentials and extension of neurites from central neurons. Our lab has demonstrated robust neurite outgrowth in embryonic trigeminal ganglia cultured with exogenous neurotrophins. We are further exploring neurotrophin roles in regulating neurite outgrowth via alterations in ion channel properties in embryonic ganglion neurons. Trigeminal ganglia are dissected from gestational day 16 rats, placed on matrix-coated coverslips, and maintained in culture with 10 ng/ml BDNF or nerve growth factor (NGF), or either neurotrophin in combination with a sodium channel blocker, tetrodotoxin (TTX) or saxitoxin (STX). Density of neurites and length of neurites are measured after 3 - 6 days in culture. Results to date indicate that BDNF - induced neurite outgrowth is reduced by STX, but not by TTX. Although the effect is less pronounced, NGF - induced neurite growth also is apparently reduced by STX. Therefore, our current results suggest that STX-sensitive and TTX-resistant sodium channels participate in neurotrophin - induced neurite outgrowth during ganglion development. Supported by NIH NIDCD Grant DC00456 to CM.

33 Poster: Development of Gustatory Systems

BDNF GENE REPLACEMENT REVEALS MULTIPLE MECHANISMS FOR ESTABLISHING NEUROTROPHIN SPECIFICITY DURING SENSORY NERVOUS SYSTEM DEVELOPMENT

Nosrat C.A.[1], Agerman K.[2], Hjerling-Leffler J.[2], Ernfors P.[2]
[1]Laboratory of Oral Neurobiology, Biologic & Materials Sciences, University of Michigan, Ann Arbor, Michigan; [2]Unit of Molecular Neurobiology, MBB, Karolinska Instituteet, Stockholm, Sweden

Neurotrophins have multiple functions during the peripheral nervous system development by controlling neuronal survival, target innervation, and synaptogenesis. Despite overlapping expression of TrkB and TrkC in many sensory ganglia, brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3) null mutant mice display selective losses in neuronal subpopulations. In the present study we have replaced the coding part of the BDNF gene with that of NT3 in mice (BDNF $^{NT3/NT3}$) to analyse the specificity and selective roles of BDNF and NT3 during development. Analysis of BDNF $^{NT3/NT3}$ mice showed striking differences in the ability of NT3 to promote survival, short-range innervation and synaptogenesis in different sensory systems. We show that NT-3 could substitute for BDNF in cochlear and vestibular systems completely or partially. However, in the gustatory system, NT3 is unable to replace the actions of BDNF possibly due to a temporally selective expression of TrkB in taste neurons, or the specific ligand expression pattern. We conclude that there is no general mechanism by which neurotrophin specificity is attained, and that specificity is achieved either by (i) a tightly controlled spatial and temporal expression of ligands, (ii) different Trk receptors playing distinct roles within the same neuronal subpopulation, or (iii) by selective receptor expression in sensory neuron subpopulations.

34 Poster: Development of Gustatory Systems

GOLGI ANALYSIS OF NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT OF NEONATAL CHORDA TYMPANI TRANSECTED RATS

Sollars S.I.[1], Engelhardt P.E.[1] *[1]Psychology, University of Nebraska, Omaha, Omaha, NE*

Transection of the chorda tympani nerve in ten-day-old rats results in permanent loss of fungiform papillae and their associated taste buds. The present study examined primary neurons in the rostral nucleus of the solitary tract (NTS) of adult rats that received chorda tympani transection at ten days of age (neoCTX) and control rats. Brainstems were sectioned and stained using a modified Golgi-Cox technique. Computerized reconstruction of stained neurons was performed on neurons found within the rostral 1/3 of the NTS. Measurements were made of somal area, dendritic length and number of dendritic branches. Analyses were performed based on the neuron type (fusiform, small and large multipolar, small and large ovoid) and surgical manipulation. Large ovoid neurons in neoCTX rats had significantly longer dendrites than large ovoid neurons in control rats. No differences between surgical groups were noted in size, length or number of branches across any of the other neuron classes. NeoCTX results in a small chorda tympani terminal field volume in the NTS. Dendritic lengthening of large ovoid neurons may be reflective of a reduction in appropriate afferent contact sites. The failure of neoCTX to affect other NTS neuronal types, despite widespread alterations in peripheral gustatory morphology, suggests that primary central gustatory neurons may be resistant to peripheral gustatory injury during development. Another possibility is that primary neurons in the NTS normally in synaptic contact with chorda tympani afferents undergo cell death following neoCTX and thus are not detectable using Golgi analysis. Supported by NIH grant NIDCD 04846.

35 Poster: Gustatory Processing

COMPARISON OF TEMPORAL PROFILES OF SINGLE FIBER RESPONSES IN CHORDA TYMPANI AND GLOSSOPHARYNGEAL NERVES IN C57BL/6J MICE

Danilov Y.[1], Danilova V.[1], Hellekant G.[1] *[1]University of Wisconsin, Madison, WI*

The purpose of the study was to characterize and compare temporal profiles of single taste fibers responses in CT and NG. In C57BL/6J mice multi-unit activity from the CT and NG was recorded during stimulation with NH4Cl, citric acid, NaCl, QHCl, denatonium (DB), sucrose and fructose. We used software-assisted spike separation technique to isolate fibers off-line. The single fiber responses during 20 s of stimulation were categorized based on temporal characteristics: 1) fast or slow development of the response, 2) phasic or tonic response and 3) inhibition. We recorded and separated 146 CT and 346 NG fibers. NH4Cl and acid elicited mainly tonic responses in both nerves, but the CT response had a fast onset and an additional phasic component versus a slow development of response in the NG. Most NaCl and QHCl CT responses were phasic versus slow and tonic in the NG. DB gave no response or inhibited the activity in the CT in contrast to slow tonic responses in the NG. Thus the later part of the response depended mainly on stimulus, whereas the development of the response was more related to the nerve: generally the responses in the CT developed faster than in the NG. However, sucrose elicited mostly responses with slow development in both nerves indicating that the slow response in NG cannot be simply attributed to the delivery of stimulus to the posterior taste buds. The difference between nerve responses might reflect involvement of different transduction mechanisms or dissimilar interactions between taste receptor cells on anterior and posterior taste buds. Supported by NIH DC005336 (V.D.)

36 Poster: Gustatory Processing

EVOKED RESPONSES TO ELECTRICAL STIMULATION OF THE GLOSSOPHARYNGEAL NERVE IN THE NUCLEUS OF THE SOLITARY TRACT IN THE RAT

Hallock R.M.[1], Di Lorenzo P.M.[1] *[1]Psychology, State University of New York at Binghamton, Binghamton, NY*

Previous work from our lab has suggested that the latency of evoked responses to electrical stimulation of the chorda tympani (CT) nerve in the nucleus of the solitary tract (NTS) can predict the response profile of a neuron. Results showed that cells with short latencies to CT stimulation were narrowly tuned to NaCl. Conversely, cells with longer latencies of response to CT stimulation were more broadly tuned. The purpose of the present experiment was to study the relationship between response profiles of NTS neurons and their evoked responses to another taste-related input, the glossopharyngeal (GP) nerve. Male Sprague-Dawley rats were anesthetized with urethane and prepared for electrical stimulation of the lingual branch of the GP nerve and for electrophysiological recording from the NTS. Once a taste-responsive cell was isolated, responses to taste stimuli (.1 M NaCl, .5 M sucrose, .01 M quinine HCl, .01 M HCl) were recorded. Next, NTS responses to electrical stimulation of the GP nerve at different frequencies/current levels were recorded. The frequencies of stimulation were 1 pulse per 20 s, 1 Hz, 20 Hz, and 50 Hz. Maximum current level was 1.5mA. Preliminary results indicate that broadly tuned cells are weakly driven by GP nerve stimulation, with relatively long latencies of 10 to 45 ms. These data support the idea that the breadth of tuning of taste-responsive cells in the NTS may be predicted by the latency of evoked responses to stimulation of their inputs. Supported by NSF grant BNS-0077965 to PMD.

37 Poster: Gustatory Processing

A SIMPLE MODEL OF A TASTE-RESPONSIVE CELL IN THE BRAINSTEM PREDICTS THE EFFECTS OF ADAPTATION

Di Lorenzo P.M.[1], Hallock R.M.[1] *[1]Psychology, State University of New York at Binghamton, Binghamton, NY*

Previous work from our lab has described the effects of adaptation of various taste stimuli on taste responses in the nucleus of the solitary tract (NTS) (Di Lorenzo & Lemon, Brain Res., 852: 383, 2000). Results showed that adaptation of certain tastants had idiosyncratic effects on NaCl-best and HCl–best cells. The present project was designed to predict these effects using a simple computational model. Inputs to the model N-best and H-best units were estimated using the response properties of taste responses in the geniculate ganglion (Lundy & Contreras, J. Neurophysiol., 82(6): 2970, 1999). These inputs were weighted based on previous work suggesting that inputs with concordant best stimuli are more effective, i.e. carry more weight, that those inputs with different best stimuli (Di Lorenzo & Monroe, Brain Res., 763: 167, 1997). Mean response profiles for NaCl-best and HCl-best units were successfully simulated using this model. The effects of adaptation were then predicted by one of two methods: 1) by eliminating the contribution of inputs whose best stimulus was the adapting stimulus, or 2) by eliminating the response to the adapting stimulus across all inputs. Results showed that overall, the first method simulated response rates that were closer to those actually recorded in the NTS following adaptation. These results suggest that adaptation may selectively affect various inputs to NTS cells rather than affecting the responses to a given stimulus across all inputs. Supported by NSF grant BNS-0077965 to PMD.

38 Poster: Gustatory Processing

CHARACTERIZATION OF EFFERENT AND AFFERENT PROJECTIONS OF CTA-INDUCED FLI CELLS IN INTS

Spray K.J.[1], Bernstein I.L.[2] *[1]Biology, Utah State University, Logan, UT; [2]Psychology, University of Washington, Seattle, Washington*

Taste aversion learning is the association between a novel taste and transient malaise such that re-exposure to the taste results in animals displaying rejection behaviors. Conditioned taste aversion (CTA) is also associated with strong fos-like immunoreactivity (FLI) in cells in the parvicellular subdivision of the intermediate nucleus of the solitary tract (iNTSpc) following exposure to a CS taste after aversion conditioning. The function of these cells is currently unknown and little is known about the neural connections to and from iNTSpc. The present studies characterized the efferent and afferent projections of CTA-induced FLI cells to better define this part of the neural circuitry underlying taste aversion learning. Several techniques were used to examine these projections: anterograde and retrograde tract tracing; double labeling for retrograde tract tracer and FLI; and, bilateral iNTSpc lesions. These studies show that FLI cells in iNTSpc receive direct projections from amygdala, paraventricular hypothalamus and lateral hypothalamus. Double labeling experiments identified the efferent targets of approximately 80% of CTA-induced FLI cells. Of these cells, over half project to structures in the pons and forebrain while the remainder project locally within the medulla. Finally, lesioning iNTSpc following acquisition of CTA show these cells are not necessary for oromotor rejection associated with expression of learning. Future studies are needed to elucidate the functional role of these projections in CTA. Supported by NIH NS37040 (ILB).

39 Poster: Gustatory Processing

SOME ROSTRAL NST NEURONS THAT EXPRESS FOS AFTER TASTE NERVE STIMULATION ARE PBN-PROJECTING NEURONS

Harrison T.[1], Rajappa P.[1], Smith-Adams L.B.[1] *[1]Anatomy & Cell Biology, East Tennessee State University College of Medicine, Johnson City, TN*

Electrical stimulation of the chorda tympani nerve (CT) or the lingual branch of the IXth cranial nerve (Ln.IX) evokes Fos expression in largely distinct populations of neurons in the rostral nucleus of the solitary tract (rNST). In the present experiments, retrograde labeling of rNST neurons from the parabrachial nucleus (PBN) was combined with electrical stimulation of these lingual taste nerves to assess the prevalence of rostrally-directed projection neurons within Fos-expressing populations of neurons. The retrograde tracer Fluorescent Gold (FG) was pressure injected unilaterally into the PBN of rats. Injections were deliberately large, typically filling the dorsolateral quadrant of the left brainstem in the PBN region, to label as many rNST projection neurons as possible. After 7-10 days survival, rats were reanesthetized and the CT or Ln.IX ipsilateral to the injection was surgically exposed for stimulation. Brain sections were processed sequentially for Fos and FG double-label immunohistochemistry, using intensified DAB and naphthol/pyronin reactions, respectively, for visualization. Initial analysis indicates that following Ln.IX stimulation, 30% of Fos-reactive nuclei on average were contained in cells with pink cytoplasm, indicating that they projected to the PBN. The majority of these double-labeled cells (61%) were located in the dorsal half of the central zone, as defined by an arbitrary counting template that divided the rNST into 4 equally sized medial-to-lateral zones. Data are being tabulated from experiments in which the CT was stimulated and results of comparisons between the two Fos-expressing populations will be presented. (Supported by NIH grant R15 NS 40265)

40 Poster: Gustatory Processing

IN VIVO INTRACELLULAR RECORDING OF GUSTATORY NEURONS IN HAMSTER SOLITARY NUCLEUS

Li C.S.[1], Li C.X.[1], Waters R.S.[1], Smith D.V.[1] *[1]Anatomy & Neurobiology, University of Tennessee Health Science Center, Memphis, TN*

Previous studies of cells in the nucleus of the solitary tract (NST) have described their gustatory response properties, morphology, and intrinsic membrane properties. Efforts to relate these parameters have met with only limited success. We have developed a preparation that allows us to record the activity of NST neurons intracellularly *in vivo* and label each cell for morphological analysis. A taste-responsive site in the NST is first identified extracellularly, At this location, an intracellular glass micropipette (120 – 150 MΩ) is inserted and the brainstem surface stabilized with a low-melting-point wax. When a stable intracellular recording is obtained, the tongue is stimulated with anodal current (50 μA, 0.5 s), followed by lingual stimulation with sucrose, NaCl, citric acid and quinine. The cell is then characterized with a current-injection paradigm (1200 ms, 100 pA, with and without a preceding 150-ms, 200-pA hyperpolarization). Cells are filled by iontophoretic injection of 2% biocytin (500 ms, 1 nA, 1 Hz, 5 – 7 min). The animal is perfused with 4% paraformaldehyde and processed for biocytin staining with DAB. We have so far recorded and filled 13 NST neurons with this protocol. Taste-sensitive cells respond to depolarizing current in a manner similar to rat NST neurons recorded *in vitro*. In two cells, stimulation of the contralateral lateral hypothalamus evoked action potentials and/or EPSPs. Histological examination revealed three different morphologies, including elongate, ovoid and multipolar cells. Supported by DC00066 to D.V.S.

41 Poster:: Gustatory Processing

PROJECTIONS OF NST NITRERGIC NEURONS

Travers S.P.[1], Shiroor C.[1] [1]*Oral Biology, Ohio State University, Columbus, Ohio*

Nitric oxide is a non-classical neurotransmitter that is gaseous and membrane permeant. The synthetic enzyme for this neurotransmitter, nitric oxide synthase (NOS), is widely but discretely localized in the CNS, indicative of specific functions. In the rostral, orosensory NST (rNST), nitrergic neurons are prominent in the area innervated by the glossopharyngeal nerve. Because the glossopharyngeal nerve plays an important role in reflex function, we hypothesized that NOS -containing neurons in rNST may be more prominently involved in gustatory-modulated reflexes than higher-order sensory function. We thus predicted that a higher proportion of rNST nitrergic neurons would project to the reticular formation or caudal NST (cNST), structures with a known involvement in oromotor and visceral reflexes, than to the parabrachial nucleus, an obligate forebrain relay. In 3 different groups of rats, iontophoretic injections of the retrograde tracer, cholera toxin, were made into the (1) medullary reticular formation, (2) cNST, or (3) parabrachial nucleus. After an appropriate survival time, rats were deeply anesthestized, perfused, and frozen sections prepared for fluorescent immunohistochemical processing to detect retrogradely- and NOS-labeled neurons. Preliminary analysis suggests that subsets of NOS neurons do project to each target. However, in each case, the proportions of nitrergic neurons that are projection neurons comprise a minority, suggesting that many NOS neurons are local interneurons. Further, NOS neurons projecting to the reticular formation or caudal NST are not more numerous than those that project to the PBN. Thus, contrary to our original hypothesis, nitrergic neurons in rNST appear functionally heterogeneous. Supported by NIH RO1DC00416

42 Poster: Gustatory Processing

MORPHOLOGY OF THE RAT PARASYMPATHETIC SECRETOMOTOR NEURONS CONTROLLING THE PAROTID AND VON EBNER'S SALIVARY GLANDS

Kim M.[1], Bradley R.M.[2], Chiego Jr D.J.[3] [1]*Nursing, Chonnam University, Gwangju, Korea;* [2]*Biologic & Materials Sciences, University of Michigan, Ann Arbor, Michigan;* [3]*Cariology, University of Michigan, Ann Arbor, Michigan*

The final common pathway of the gusto-salivary reflex arc is via parasympathetic neurons of the salivatory nuclei. The inferior salivatory nucleus supplies both the parotid (PG) and Von Ebner's (VE) lingual salivary glands. Neurons supplying the VE and PG were separately labeled in the same animals. The VE gland neurons were retrogradely labeled by Alexa Fluor dextran 568 (red) applied to the glossopharyngeal nerve, while the PG neurons were labeled with Alexa Fluor dextran 488 (green) injected into the otic ganglion. Horizontal, 100 micron brainstem sections were subsequently viewed in a confocal microscope and a stacked series of 1 micron images collected. Ten individual neurons supplying the VE and ten supplying the PG were then traced and analyzed using Neurolucida image analysis software. Neurons supplying the two glands form distinct and separate populations. The VE neurons are located dorsal and rostral to the PG neurons. Several measures of neuron morphology were made, including soma area and form factor, number of primary dendrites and dendritic segments and total length. Neurons innervating the PG are larger than those innervating VE. Furthermore, all morphometric measures of the two populations were significantly different except soma form factor. Supported by NIH grant DC000288 to RMB.

43 Poster: Gustatory Processing

BIOPHYSICAL AND MORPHOLOGICAL PROPERTIES OF BRAINSTEM PARASYMPATHETIC NEURONS CONTROLLING VON EBNER'S GLANDS

Fukami H.[1], Chiego Jr. D.J.[1], Bradley R.M.[1] [1]*Biologic & Materials Sciences, University of Michigan, Ann Arbor, Michigan*

Von Ebner's lingual salivary glands secrete saliva into the clefts of the circumvallate and foliate papillae. Stimulation of taste receptors lining the clefts of these papillae results in reflex secretion of saliva which then influences taste responses recorded from circumvallate and foliate taste buds (Gurkan and Bradley. Chem.Senses 13:655, 1988). To clarify the mechanisms underlying this gusto-salivary reflex, we have recorded from identified secretomotor neurons innervating von Ebner's gland. Neurons were retrogradely labeled with fluorescent Alexofluor dextran and whole cell patch clamp recordings made from labeled neurons in brainstem slices. Neurons were first visualized with fluorescent illumination and once a neuron was selected for recording, it was imaged using differential interference contrast infra-red microscopy. The electrode also contained Lucifer Yellow (LY) to fill the recorded neuron for subsequent identification. Successful recordings have been made from 45 neurons. Using various current injection protocols four groups of neurons have been defined based on biophysical and repetitive discharge characteristics. After recording the brain slice was fixed and cleared, the LY filled neuron was imaged using confocal microscopy and then reconstructed. Based on various morphometric measures no distinct morphological groups of neurons were found. The neurons form a continuum of different morphological characteristics that vary quantitatively. Supported by NIH grant DC000288 to RMB.

44 Poster: Gustatory Processing

TASTE, TEXTURE AND TEMPERATURE REPRESENTATION IN THE PRIMATE ROSTRAL INSULAR AND FRONTAL OPERCULAR CORTEX

Kadohisa M.[1], Rolls E.T.[1], Verhagen J.V.[1] [1]*Exp Psychology, University of Oxford, Oxford, United Kingdom*

The rostral insular and frontal opercular cortex in primates contains the primary taste cortex. We investigated whether neurons in this cortical area have oral somatosensory responses, and whether olfactory and visual inputs reach this area in the rhesus macaque. Single neurons were tested with 4 prototypical taste stimuli (1M glucose, 0.1M NaCl, 0.01M HCl, and 0.001M quinine HCl), 0.1M MSG, distilled water at 10°C, 23°C, 37°C and 42°C and a 1 log unit spaced viscosity series in the range 1-10,000 centiPoise made of carboxymethylcellulose. Of the 57 neurons tested to these stimuli, 15 (26%) responded to only gustatory stimuli, 3 (5%) responded to only textual or thermal stimuli. Eleven (19%) responded to both taste and texture or to both taste and temperature, 4 (7%) responded to both texture and temperature. Five neurons (9%) responded to taste, temperature and texture. Of 15 neurons that responded to gustatory stimuli, none responded significantly to olfactory stimuli or to visual stimuli which included the sight and smell of the monkey´s favorite food. These results show that some neurons in the insular and frontal opercular primary taste cortex (as defined by thalamic input) respond to combinations of taste with oral temperature and texture. Further, these neurons do not respond visual and olfactory inputs, a type of convergence which is left until the secondary taste cortex in the orbitofrontal cortex (Rolls, E.T. and Baylis, L.L. 1994 Gustatory, olfactory and visual convergence within the primate orbitofrontal cortex, Journal of Neuroscience 14: 5437-5452).

45 **Poster: Gustatory Processing**

OTITIS MEDIA INFLUENCES BODY MASS INDEX BY INTERACTING WITH SEX, AGE, AND TASTE PERCEPTION

Snyder D.J.[1], Duffy V.B.[2], Chapo A.K.[1], Hoffman H.J.[3], Bartoshuk L.M.[1] *[1]Surgery, Yale University, New Haven, CT; [2]Dietetics, University of Connecticut, Storrs, CT; [3]NIDCD, Bethesda, MD*

Obesity is a major health concern for people of all ages; otitis media (OM) is a common childhood disease that may persist in adults. Since the chorda tympani (VII) passes through the middle ear, OM can damage it and reduce taste sensation; since VII inhibits other orosensory inputs (V, IX) centrally, OM damage can also alter oral touch/irritation and retronasal olfaction. Oral sensation guides food preference/intake, thereby affecting body mass index (BMI). Here we present evidence that OM may lead to health risk by contributing to increased BMI. Survey participants (N=3876) answered questions about their sex, age, height/weight, and OM history; they also rated the intensity of filter papers containing 6-n-propylthiouracil (PROP). Multiple regression revealed significant positive effects of OM, sex, age, and PROP on BMI. In particular, men over age 30 with a history of OM had significantly higher BMIs than those without. Further, while BMI usually falls with rising PROP intensity, in this group of men aged 30+, supertasters (ST) had higher BMIs than nontasters. There were no OM effects on BMI for women. Thus, men over age 30 with OM history (especially STs) are at higher risk for obesity. By reducing taste input from VII, OM may alter fat sensation and preference via release of inhibition on the trigeminal nerve. In some cases, increased fat perception in men produces increased fat preference; this could lead to increased fat intake and BMI. STs, who receive the most intense taste and trigeminal input, may experience greater increases in fat sensation due to greater release of inhibition. We conclude that OM significantly influences BMI and obesity risk in men. (DC 00283)

46 **Poster: Sweet Taste**

BEHAVIORAL, NEURAL AND MOLECULAR GENETIC STUDIES ON THE DPA (D-PHENYLALANINE SENSITIVITY) LOCUS IN MICE

Shigemura N.[1], Nakashima K.[2], Kawato Y.[3], Ninomiya Y.[1] *[1]Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan; [2]Department of Chemistry, Asahi University School of Dentistry, Gifu, Japan; [3]Bio-oriented Technology Research Advancement Institution (BRAIN), Saitama, Japan*

Two loci on mouse chromosome 4, *dpa* and *Sac* (Saccharin preference) have been proposed to influence responses to sweet substances. Recently, the *Sac* locus is found to be a candidate gene encoding a G-protein-coupled sweet receptor, T1R3. However, no such progress has been made for the dpa locus. In this study, we investigated possible action of the *dpa* locus on various levels of the taste system by using the *dpa* congenic strain (CG) having BALB background except a gene segment including the *dpa* locus derived from D-phenylalanine responsible C57 strain. In both CG and C57, unlike BALB, a conditioned taste aversion (CTA) of D-Phenylalanine generalized to 0.1 M sucrose. Behavioral analysis using the CTA paradigm demonstrated that sensitivities to gurmarin (a specific inhibitor for sweetener responses) and beta-cyclodextrin (a reducer for the effects of gurmarin) were similarly observed in responses to sweet substances in both CG and C57 (gurmarin-sensitive strain), but not in BALB (gurmarin-insensitive strain). This indicates a possibility that the transferred gene segment in CG may contain a gene influencing gurmarin-sensitivity at the site of the taste receptor cell membrane. Our subsequent molecular genetic studies further examined the candidate *dpa* transduction elements in CG by using the cDNA subtraction and other possible methods.

47 **Poster: Sweet Taste**

NOVEL TRANSDUCTION PATHWAY FOR THE ARTIFICIAL SWEETENER, ACESULFAME-K, POTENTIALLY UTILIZES A TRP MEDIATED CHLORIDE CURRENT.

Zhao F.[1], Lu S.[1], Herness S.[1] *[1]College of Dentistry, Ohio State University, Columbus, OH*

Transduction pathways for sweet stimuli involve either the cyclic AMP or the IP_3 pathway. Using patch-clamp and calcium imaging techniques on rat taste receptor cells (TRCs), we report a novel pathway for the artificial sweetener acesulfame-K that potentially involves activation of a TRP channel and subsequent activation of a chloride current. Acesulfame-K (10 mM) enhances a chloride current specifically in TRCs expressing both sodium and potassium currents. This current is unaffected by other stimuli such as saccharin, cycloheximide, or denatonium. Replacement of barium for calcium blocks this effect, suggesting this is a calcium-dependent chloride current. Blockers of the IP_3 pathway were without effect. Using fura-2, acesulfame-K was also observed to increase intracellular calcium in approximately 30% of TRCs. We were able to measure an inward current directly induced by acesulfame-K. This current did not have characteristics of voltage-gated calcium current. SKF 96365, a specific blocker of store operated channels, did not block the inward current. The PLC blocker U73122 could enhance this current in a subset of cells, which suggests that PIP_2 might increase the current. Lanthanum, a nonspecific blocker of many TRP channels, was without effect. These results suggest the current is composed of an inward calcium component and an outward chloride component. Since other stimuli that elevate intracellular calcium do not increase the calcium-activated chloride current, a tight linking between calcium influx and chloride amplification is proposed through an agonist-operated rather than store-operated calcium influx. This work was supported by NIH DC00401.

48 **Poster: Sweet Taste**

STARCH-BASED FLAVOR FILMS: A NOVEL METHOD FOR WHOLE MOUTH STIMULATION WITH PRECISE STIMULUS AMOUNTS

Flammer L.J.[1], Sondrup J.[2], Grainger B.[2], Alvarez-Reeves M.[3], Green B.[4] *[1]Global Sensory & Consumer Science, International Flavors & Fragrances, Inc., Union Beach, NJ; [2]International Flavors & Fragrances; [3]John B. Pierce Laboratory, New Haven, CT; [4]John B. Pierce Laboratory and Yale School of Medicine, New Haven, CT*

Typical modes of taste stimulus delivery (e.g., sip-and-spit, filter paper) do not mimic actual tasting. Thin, starch-based flavor films similar to commercial breath films potentially provide a more natural yet well-controlled method for delivering precise quantities of gustatory stimuli. We compared this method to two others that also enable delivery of precise quantities under conditions of normal tasting: micropipetting of aqueous solutions onto the tongue, and licking equivalent amounts of pure, dried stimulus from a non-absorptive surface (Parafilm). The effectiveness of the three methods was compared by asking 12 Ss to rate the perceived taste intensity (sweet, sour, salty, bitter, and other) of four quantities of saccharin (5.6, 10.2, 18.1 and 31.7μg) plus a blank containing no saccharin. Micropipetting and licking produced nearly identical psychophysical functions for sweetness, whereas the films produced significantly higher intensity ratings for the lower stimulus quantities. Although sweetness ratings for the blank films were less than 'barely detectable' on the Labeled Magnitude Scale, subsequent pilot tests with the nose pinched vs. nose open indicated the films produced a very weak olfactory sensation that may have enhanced sweetness via an olfactory-taste interaction. Research is continuing to quantify this striking taste enhancement, and to explore use of the films as a new and sensitive method for studying olfactory-taste interactions under conditions of normal tasting. Supported in part by NIH Grant DC05002

49 Poster: Sweet Taste

SWEET AND SOUR PREFERENCES IN YOUNG CHILDREN AND ADULTS: ROLE OF REPEATED EXPOSURE

Liem D.[1], Van Den Elshout J.[1], Lam K.[1], De Graaf K.[1] *[1]Human Nutrition, Wageningen University, Wageningen, Netherlands*

We investigated the influence of repeated exposure to beverages varying in sweetness and sourness, on the taste preferences of 6-to-9-year-old children and young adults. During an exposure period of 8 days, 59 children (9.2± 0.9 yrs.) and 46 young adults (22±2.0 yrs.) received either a sweet orange flavored beverage (0.42M sugar; 0.0 M citric acid), sour beverage (0.42M sugar; 0.065M citric acid), or no beverage (control). Before and after the exposure, preference for a series of sweet beverages (0.42M sucrose) with different concentrations of citric acid (0.009M; 0.013M; 0.020M; 0.029M; 0.043M; 0.065M) and sweet yogurt (0.42M sucrose; 0.027M; 0.038M; 0.056; 0.081M; 0.12M; 0.17M added citric acid) was measured by means of a rank ordering procedure. The results suggest that, after an 8-day exposure to the sweet beverage, children´s, but not adults´ preferences for the sweetest beverage significantly increased ($p<0.05$). A similar trend was observed for the sweetest yogurt ($p=0.09$). No difference in preference was observed for those children and adults who were exposed to the beverage with a high concentration of citric acid. As expected the control group of both children and adults did not show any change in preference. In conclusion, sweet, but not sour preferences in children, but not adults can be heightened by repeated exposure. Future research is needed to investigate the influence of repeated exposure to sweet and subsequent consumption of sweet food items.

50 Poster: Sweet Taste

INDIVIDUAL DIFFERENCES IN SWEET PREFERENCES ACROSS THE LIFESPAN

Pepino M.Y.[1], Kennedy J.M.[1], Currier M.P.[1], Mennella J.A.[1] *[1]Monell Chemical Senses Center, Philadelphia, PA*

Preference for sweet tastes is heightened during infancy, childhood and adolescence. Although studies conducted in the 1970´s suggested the existence of ethnic differences in sweet preferences during adolescence, it is not known whether similar differences are present during childhood and adulthood. Therefore, the present study aimed to determine the preferred level of sucrose in 5- to 10-year-old children and their mothers by using a forced-choice, paired comparison, tracking procedure. Approximately half of the children (46%) were Black whereas 40% were White as reported by both parents; there was no significant difference in income levels between the two groups. Consistent with previous findings on ethnic-related differences in preference for sweets during adolescence, Black children preferred significantly higher concentrations of sucrose than did White children. Although mothers preferred lower concentrations than children, a similar ethnic-related difference in sweet preferences was also observed in them. Moreover, ethnic differences existed in the practice of feeding sugar water to infants and those Black children who were routinely fed sugar water as infants exhibited heightened preferences for sweet tastes when compared to those without such experience. Finally, regardless of group, children who preferred higher concentrations of sucrose were significantly more likely to report they added sugar to their foods and that they preferred sweet to salty snacks. Whether these differences in sweet preferences are due to experiential or physiological differences and whether they are related to the elevated incidences of obesity and diabetes among Black children is not known. Supported by NIH Grants AA09523 and HD37119.

51 Poster: Sweet Taste

APPARENT SPECIFIC VOLUMES (ASVS) OF CYCLAMATES AND OTHER SULFAMATE SWEETENERS

Haywood K.A.[1], Spillane W.J.[2], Hanniffy G.[2], Coyle C.[3], Birch G.G.[4] *[1]Food Biosciences, Reading, U.K.; [2]Chemistry, NUI, Galway, Ireland; [3]Chemistry, NUI, Galway, Ireland; [4]Food Biosciences, University of Reading, Reading, U.K.*

Cyclamate (cyc-$C_6H_{11}NHSO_3M$) sweeteners continue to attract interest for many reasons not least being that they are good at synergy, are relatively inexpensive to produce and the calcium and sodium (M = Ca, Na) salts are permitted sweeteners in the EU and many countries worldwide. The measurement of apparent specific volumes (ASVs) has given a unique insight into the mode of action of cyclamates and of other tastants. In the present work we have synthesized and measured ASV values for various cyclamates and other sulfamates ($RNHSO_3Na$) and when these are taken together with data previously presented for a series of cyclamates and sulfamates a total data base of 34 ASV values is now available. When all 34 ASV values are presented in a Figure not unexpectedly most cluster in the 'sweet ASV region' i.e. ~ 0.5 to ~0.7. However in order to embrace all the sweet cyclamates/sulfamates this region needs to be extended to ~0.45. A few cyclamate salts namely, thallium and barium fall well below this region and may in fact be sour. Taste data on some new compounds will be presented. Three sodium anilinomethanesulfonates ($XC_6H_4NHCH_2SO_3Na$) have ASVs in the sweet region but, interestingly, preliminary taste data indicates that they are not sweet, thus showing that though the ASV values of these three compounds are ideal for sweetness the molecular structural change made (compared to the parent $-NHSO_3$ - moiety) cannot be accommodated at the receptor site. Finally following on from our previous presentation some cyclamate cation effects will be discussed using the data now available for 16 salts.

52 Poster: Bitter Taste

SOLUTION PROPERTIES AND THE ROLE OF WATER IN THE INHIBITION OF BITTERNESS IN MIXTURES OF SWEET AND BITTER MOLECULES

Portmann M.[1], Aroulmoji V.[2], Liu J.[3], Mathlouthi M.[2] *[1]NPD, GlaxoSmithKline Consumer Healthcare, Weybridge, Surrey, United Kingdom; [2]Laboratoire de Chimie Physique Industrielle, Universite de Reims, Reims, France; [3]NPD, GlaxoSmithKline Consumer Healthcare, Parsippany, NJ*

Solution properties (intrinsic viscosity, apparent specific volume (ASV) and surface tension &gamma) of caffeine and sweeteners (sucrose, glucose, fructose) and their mixtures were determined with the aim of finding arguments supporting the role of water structure in the mechanism of taste chemoreception. Hydrophilic carbohydrates have ASVs within 0.52-0.71 cm3g-1 whereas caffeine has an ASV of 0.937 cm3g-1 due a hydrophobic surface which does not fit with water structure. &gamma value shows a slight increase with carbohydrates but the detergent effect of caffeine provokes a decrease of &gamma& from 73.5 to 70.1 mN/m. Solution properties of caffeine-carbohydrate mixtures show an improvement of the fitting of solute molecules with solvent structure through the decrease of ASV which comes nearer to the range of ASVs of sweet molecules. The lower &gamma is interpreted as an enhancement of adsorption of bitter molecules on hydrophobic receptor membrane. The effect of sweeteners on the solution properties of bitter molecules can be described as a modification of hydration to make it comparable to that of sweet molecules. This tends to allow qualification of sweeteners as bitterness inhibitors. The mechanism of inhibition of bitterness by sweet molecules is likely due to the rearrangement of hydration water molecules which become more mobile.

53 **Poster: Bitter Taste**

BITTERNESS INHIBITION OF BINARY MIXTURES OF BITTER COMPOUNDS BY SODIUM SALTS

Canty T.M.[1], Keast R.S.[1], Breslin P.A.[1] [1]*Monell Chemical Senses Center, Philadelphia, PA*

Sodium salts suppress the bitterness of certain compounds. To date all investigations of bitterness suppression in model aqueous systems have used only single bitter compounds. This study investigates the influence of a variety of sodium salts on bitterness suppression of binary mixtures of bitter compounds. Subjects (n=15) were screened for their ability to identify basic taste qualities and were trained in the use of the general Labeled Magnitude Scale. Five bitter compounds were used: L-tryptophan, Tetralone (iso-alpha acid mixture), denatonium benzoate, quinine-HCl, and Ranitidine. Only the following binary combinations were used: Denatonium-Ranitidine, Tetralone-Ranitidine, Tryptophan-Tetralone, Tetralone-Denatonium, Tryptophan-Quinine, and Ranitidine-Quinine. Each compound was tested at a predetermined concentration to elicit bitterness between `weak´ and `moderate´. These concentrations were combined to form the binary mixtures, and single compounds were tested at double concentrations as a control. The following sodium salts (100 mM Na+) were added as putative bitterness inhibitors: Sodium chloride, sodium gluconate, monosodium glutamate, and adenosine monophosphate sodium salt. Each of the bitter stimuli were tasted with and without each sodium additive. In general, bitterness inhibition of binary bitter combinations by sodium could be predicted from the average bitterness suppression of the individual components. Implications for sodium´s influence on the bitter taste system and how bitter stimuli combine will be discussed. Supported by Firmenich grant to RSJK and PASB and NIHDC 02995 to PASB.

54 **Poster: Bitter Taste**

PERCEPTION OF BITTERNESS FROM CAPSAICIN, PIPERINE AND ZINGERONE

Green B.[1], Hayes J.[1] [1]*John B. Pierce Laboratory, New Haven, CT*

It was recently found that in some people, capsaicin evokes bitterness as well as irritation, especially on the back of the tongue. One possible explanation is that in such individuals, some "bitter-sensitive" taste receptor cells express the vanilloid receptor VR1. If so, other vanilloids should also evoke bitterness, and the incidence of bitterness should be correlated with the bitterness of capsaicin. We tested this hypothesis by presenting capsaicin and its congeners, piperine and zingerone, to the circumvallate (CV) papillae in a group of Ss who had been screened for reliable access to that region. Prior to rating the chemesthetic stimuli, Ss rated 4 classical gustatory stimuli (sucrose, citric acid, NaCl and QHCl) delivered to the CV papillae via cotton swabs. The vanilloid stimuli, which were presented in random order in separate sessions, were prepared by dipping swabs in ethanol solutions (100μM capsaicin, 70mM piperine, and 296mM zingerone), allowing the swabs to dry, then wetting them with dH20 before application. Among 24 Ss who were tested, pair-wise correlation analyses showed that bitterness ratings for capsaicin were significantly related to bitterness ratings for piperine and zingerone (both Pearson r´s = +0.69); the relationship between the bitterness of piperine and zingerone was even stronger (r = +0.92). 13 of the 24 Ss (54.2%) reported at least `weak´ bitterness for 1 or more of the vanilloids, and 7 of the 13 did so for all three. The results therefore suggest that a common transduction mechanism, possibly VR1, mediates the perceived bitterness of different vanilloids. (Supported by NIH grant DC05002)

55 **Poster: Bitter Taste**

SENSITIVITY FOR PROP AND BASIC TASTES IN YOUNG AND ELDERLY MALE AND FEMALE SUBJECTS

Mojet J.[1] [1]*Agrotechnologisch Research Institute (ATO), Wageningen, Netherlands*

What is the relationship of the sensitivity to the bitter tasting compound PROP (6-n-propylthiouracil) with the sensitivity for other bitter and non-bitter tasting compounds and how do age and gender influence this? PROP-sensitivity was determined with two methods (filter paper and 3 times PROP-NaCl ratio) in a group of 21 young (10 female, 11 male; age 19-33 yrs) and 21 elderly (11 female, 10 male; 60-75 yrs) subjects, whose taste thresholds (6 times 2 AFC-5 in a row) and suprathreshold sensitivity (3 times intensity rating) for 10 tastants (2 for each taste quality) had been determined (Mojet et al, 2001, 2003). Both PROP-methods correlated highly ($r=0.75$, $p=0.0001$). GLM-analysis (factors: age and gender; dependent variables: prop-ratio, threshold sensitivity, intensity in water and product, and pleasantness) showed that the young (especially the females) were significantly less sensitive ($p<0.0001$) to PROP than the elderly. In contrast, a significant overall age effect in favour of the young was found for both the threshold ($p<0.003$) and supra-threshold measurements (water: $p<0.0001$, product: $p<0.03$) with the ten tastants. No significant correlations between PROP sensitivity and the thresholds, the perceived intensities in water and in product, or the pleasantness in product are found for either the bitter tastants KCl, caffeine and quinine HCl, or the non-bitter tastants NaCl, sucrose, aspartame, acetic acid, citric acid, MSG and IMP. These results suggest that the mechanisms involved in the perception of PROP and of the other tastants are essentially different.

56 **Poster: Bitter Taste**

GENETIC STUDIES OF PTC TASTE IN HUMANS

Drayna D.[1], Kim U.[2], Jorgenson E.[3], Risch N.[3], Coon H.[4], Leppert M.[5] [1]*National Institutes of Health, Rockville, MD;* [2]*NIDCD/NIH, Rockville, MD;* [3]*Genetics, Stanford University, Stanford, CA;* [4]*Psychiatry, University of Utah, Salt Lake City, UT;* [5]*Genetics, University of Utah, Salt Lake City, UT*

We used a forced-choice blind sorting test to determine PTC taste thresholds in large, genetically well-characterized families within the Utah Genetic Reference Project. Taste thresholds showed a continuous bimodal distribution, and genetic linkage studies using taste threshold as a quantitative trait revealed linkage to markers on the distal portion of chromosome 7q, near the KEL blood group antigen gene, as well as to other loci in the genome. In families showing linkage to chromosome 7q, cross-over events have delimited the location of the gene to a small interval in the vicinity of the marker D7S661. We performed a high-resolution genetic analysis of this region, and we have observed strong linkage disequilibrium between taster status and alleles of markers within this interval. This finding suggests that apparently unrelated individuals who are non-tasters due to the action of the chromosome 7 gene share a founder mutation identical by descent. DNA sequence analysis of candidate genes within the region of linkage disequilibrium suggests a single gene contains sequence differences that account for PTC taste phenotype.

57 Poster: Bitter Taste

PROP BITTERNESS ASSOCIATES WITH DIETARY FAT BEHAVIORS AND RISK FOR CARDIOVASCULAR DISEASE (CVD) IN MIDDLE-AGED WOMEN

Hutchins H.L., Healy N.A., Duffy V.B. *Univ. Connecticut, Storrs, CT*

This study continues our investigation of genetic variation in taste and CVD risk. Thirty-four females (mean age=49±1 SE years) used the general labeled magnitude scale to rate PROP bitterness, NaCl saltiness, as well as creaminess of and preference for sampled high-fat foods. Subjects were interviewed on how frequently they consumed core high-fat foods over the past year using the Block Food Frequency instrument. Risk of CVD was assessed by measures of adiposity, resting blood pressure and fasting serum lipids for total cholesterol, HDL- and LDL-subfractions. Those who tasted the bitterness of .0032 M PROP as greater than "moderate" reported significantly greater creamy sensations from and less liking for added fats (heavy cream, mayonnaise, cream cheese). The ratio of PROP bitterness to NaCl saltiness (Bartoshuk et al, 1994) showed stronger relationships with diet and CVD risks. Women who had higher PROP ratios reported less frequent intake of 23 of the 28 foods that were consumed most frequently across all subjects. These 23 foods formed a statistically reliable group (Cronbach's alpha=0.68). PROP ratio was significantly lower in women who were: overweight or obese (body mass index>25) and had central adiposity (waist circumference>35 inches); had the potential for high blood pressure (systolic pressure>120 mmHg or diastolic pressure>80 mmHg); and had an elevated total cholesterol (>200 mg/dl) and LDL subfraction (>130 mg/dl). These data support that those who taste PROP as least bitter may have greatest CVD risk and that the relationship appears to be mediated in part through oral sensations from fat and dietary behaviors toward high-fat foods. (NRICGP/USDA 2002-00788 funded)

58 Poster: Trigeminal Chemoreception & Irritation

REAL-TIME MONITORING OF NASAL MUCOSAL PH DURING CO_2 STIMULATION: A PILOT STUDY

Avila P.C., Shusterman D.J. *Univ. California, San Francisco*

Carbon dioxide is a commonly employed irritant test compound in nasal chemesthetic studies because it is essentially free of olfactory stimulus properties. CO_2 is thought to act via hydration to H_2CO_3 and dissociation to H^+ in nasal mucus, with resulting activation of acid sensors. Indirect evidence for this mechanism of action comes from studies in which carbonic anhydrase is inhibited with a resulting reduction in electrophysiologic response. However, at least in the published literature, transient changes in nasal mucosal pH have not been documented during CO_2 stimulation in humans. We placed a small pH probe on the floor of the right anterior nasal cavity during CO_2 stimulation in 8 subjects (6 males and 2 females) with either consistently high (> 35%) or low (< 20%) CO_2 detection thresholds (4 in each subgroup). 3-sec. pulses of CO_2 (15-45% vol/vol) paired with air in random order (12-15 sec. ISI; 60 sec. ITI) were administered by nasal cannula at 5 L/min in an ascending series. For each subject, both a CO_2 detection threshold and a psychophysical rating curve [labeled magnitude scale] were generated ("threshold" being defined as 5/5 correct responses at a given stimulus level). All subjects showed phasic drops in pH associated with CO_2 stimulation. Latencies to peak deviation varied from 2.5 to 30 sec, and the magnitude of pH change ranged up to 0.31 pH units. For all subjects combined, a positive correlation was apparent between delta pH and psychophysical rating of nasal irritation ($p < 0.001$). This technique holds promise for the documentation of both pre- and peri-receptor events in nasal chemesthesis when CO_2 is the test irritant employed.

59 Poster: Trigeminal Chemoreception & Irritation

CARBONIC ANHYDRASE GENE EXPRESSION IN THE HUMAN NASAL MUCOSA: A PILOT DESCRIPTIVE STUDY

Tarun A.[1], Bryant B.[2], Shusterman D.J.[1] [1]*Medicine, University of California, San Francisco, San Francisco, CA;* [2]*Neurophysiology, Monell Chemical Senses Center, Philadelphia, PA*

Carbonic anhydrase (CA) is physiologically important in the reversible hydration and dissociation of CO_2; it is expressed in a number of isoforms with varying enzymatic activities (CA I-XIV). In oral chemesthesis, CA is essential for the normal sensation of "carbonation," and in nasal chemesthesis, CA inhibition decreases the electrophysiologic response to CO_2 (a commonly employed irritant test compound). CA enzymatic activity has been demonstrated in the human nasal mucosa using enzyme histochemical methods, but no systematic study of nasal mucosal CA isoenzyme gene expression has been published. To address this question, we examined CA gene expression in superficial nasal mucosal scrapings from four subjects (3 females and 1 male; age range, 28-54 years). RNA was extracted from the nasal scrapings and reverse transcribed to generate cDNA. Non-quantitative PCR was performed using primers for each of the 11 active CA isoenzyme genes and the housekeeping gene GADPH. Amplification products of GADPH and 10 of the 11 CA genes were detected in most specimens (CA VI was detected in 3 of 4, and CA VA was not detected). In addition, we observed variable intensities for the amplification products for CA IV, VI, VII and IX. Our data demonstrate inter-individual variability in the expression of CA isoenzymes in human nasal mucosa, potentially accounting for differences in CO_2 nasal chemosensitivity between individuals. We are currently testing this hypothesis by determining the quantitative expression of CA isoenzyme genes using real-time PCR from RNA extracted from individuals with known CO_2 detection threshholds.

60 Poster: Trigeminal Chemoreception & Irritation

IDENTIFICATION OF TRIGEMINAL SUBPOPULATIONS DIFFERING IN THEIR P2X-RECEPTOR EXPRESSION

Paul J.[1], Spehr M.[1], Hatt H.[1], Wetzel C.H.[1] [1]*Ruhr-University Bochum, Bochum, Germany*

Trigeminal nerve fibers innervating the facial mucous membranes are sensitive to chemical stimuli with the primary function of protection from harmful chemicals. Additionally, the trigeminal system can mediate odour sensations as shown psychophysically in anosmic humans. It has been suggested recently, that the neuromodulator ATP modulates odour responsiveness of ORNs by activating purinergic receptors. We investigated the expression of purinergic receptors (P2X) in the trigeminal system by using dissociated cultured neurons of the rat gasserian ganglion. ATP-induced currents were characterized using the patch clamp technique and showed three different time courses of the response: sustained, transient and mixed. We pharmacologically identified three subpopulations of neurons expressing different subtypes of P2X-receptors: Neurons showing sustained currents likely express homomeric P2X2 receptors, the subpopulation showing transient currents expresses homomeric P2X3 receptors. The mixed current was probably based on the expression of two different populations of homomeric P2X2 and P2X3 receptors. Immunostaining with subunitspecific antibodies supported the pharmacological data and revealed expression of P2X receptors on the somata and fibres. The functionality of these receptors located on the trigeminal fibres could be shown in Ca-Imaging experiments by local ATP-superfusion. The role of the purinergic receptors in the perception of chemical stimuli like odorants is still under investigation.

61 Poster: Trigeminal Chemoreception & Irritation

LACK OF QUININE-EVOKED ACTIVITY IN RAT TRIGEMINAL SUBNUCLEUS CAUDALIS

Simons C.[1], Boucher Y.[2], Carstens E.[1] [1]*Neurobiology, Physiology and Behavior, University of California, Davis, California;* [2]*UFR Odontologie, Universite Paris 7, Paris, France*

Conflicting reports exist regarding the ability of quinine to activate neurons in the trigeminal system. We used the complementary approaches of single-unit electrophysiology and c-fos immunohistochemistry to investigate whether quinine (0.1 M) activates chemonociceptive cells in the brain stem trigeminal subnucleus caudalis (Vc). In electrophysiological experiments using pentobarbital-anesthetized rats, 31 units responded to noxious mechanical, thermal and chemical (0.2 M pentanoic acid) stimuli applied to the tongue with an increase in firing rate. None of the units responded to oral quinine. Moreover, application of quinine to the dorsal anterior tongue did not elicit significantly higher levels of fos-like immunoreactivity (FLI) in dorsomedial Vc compared to vehicle (distilled water). These data indicate that quinine does not elicit activity in chemonociceptive Vc neurons. This work was supported by grants from the California Tobacco-Related Disease Research Program, # 10DT-0197, 11FT-0101 and 11RT-0053, the National Institute of Dental and Craniofacial Research, # DR13685, and the International Association for the Study of Pain.

62 Poster: Trigeminal Chemoreception & Irritation

TRIGEMINAL RESPONSES TO BITTER-TASTING SUBSTANCES APPLIED TO THE NASAL CAVITY

Silver W.L.[1], Alimohammadi H.[1], Anderson K.T.[2], Bottger B.[2], Hansen A.[2], Finger T.E.[2] [1]*Biology, Wake Forest University, Winston-Salem, NC;* [2]*Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO*

Inhalation of many volatile substances leads to activation of nasal trigeminal nerve endings, triggering protective reflexes such as coughing and sneezing. The nasal epithelium is richly innervated by free nerve endings of the trigeminal nerve and the free nerve endings are thought to mediate responses to these volatiles. In addition, we recently reported the existence of an extensive population of solitary chemosensory cells scattered across the respiratory epithelium and innervated by peptide-containing fibers of the trigeminal nerve. These solitary chemosensory cells express alpha-gustducin as well as some of the T2R family of receptors which in taste buds mediate responses to bitter-tasting substances. Since the SCCs contain the molecular machinery suitable for detection of bitter-tasting substances, we tested whether the trigeminal nerve is responsive to stimulation by prototypical non-volatile bitter compounds. Multi-unit recordings were obtained from the ethmoid branch of the trigeminal nerve in rats, in response to perfusion of the nasal cavity by denatonium, quinine and cycloheximide. Results demonstrate clear trigeminal nerve responses to all of these, often accompanied by acute depressions in respiratory rate. Furthermore, both the nerve responses and the respiration effects are absent in neonatally capsaicin-desensitized animals, suggesting that these chemosensory cells are specifically innervated by a subgroup of trigeminal fibers expressing the vanilloid receptor.

63 Poster: Trigeminal Chemoreception & Irritation

CONTRIBUTION OF VANILLOID RECEPTOR-EXPRESSING FIBERS TO OVERALL TRIGEMINAL NERVE CHEMOSENSITIVITY

Alimohammadi H.[1], Silver W.L.[1] [1]*Department of Biology, Wake Forest University, Winston-Salem, North Carolina*

Capsaicin-sensitive, vanilloid receptor (VR1) expressing trigeminal ganglion neurons form a major subgroup of nociceptive fibers innervating the nasal mucosa. In rats, elimination of these fibers through neonatal capsaicin treatment has been shown to produce adults with decreased trigeminal sensitivity. To investigate further the contribution of VR1-expressive nociceptors to overall nasal trigeminal chemosensitivity, adult Sprague-Dawley rats injected with capsaicin as neonates were subjected to a series of behavioral and electrophysiological experiments. Capsaicin-treated rats were found to display significantly decreased aversion reactions to amyl acetate, cyclohexanone, ethanol, and nicotine, all of which are non-acidic. In contrast, acetic acid produced strong reflex rejection movements in both control and capsaicin-treated animals. Following behavioral experiments, multi-unit recordings were obtained from the ethmoid nerves of the same rats in response to a series of irritants presented via an air-dilution olfactometer. In accord with the results of the behavioral experiments, capsaicin-treated rats displayed significant nerve responses only to acetic acid and carbon dioxide. While the present results do not rule out acid-sensitivity of VR1-expressing fibers, they do indicate that a significant portion of trigeminal nerve sensitivity to the acidic stimuli tested is mediated by fibers not expressing the vanilloid receptor. These data also indicate that the same group of capsaicin-sensitive fibers mediates nasal trigeminal sensitivity to the non-acidic stimuli tested.

64 Poster : Trigeminal Chemoreception & Irritation

INVESTIGATION OF THE CENTRAL TOPOLOGY OF SENSORY TRIGEMINAL FIBERS INNERVATING THE NASAL MUCOSA

Damann N.[1], Klopfleisch R.[2], Teifke J.P.[2], Klupp B.[2], Hatt H.[1], Mettenleiter T.C.[2], Wetzel C.H.[1] [1]*Lehrstuhl fuer Zellphysiologie, Ruhr-Universitaet, Bochum, Germany;* [2]*Friedrich-Loeffler-Institute, Bundesforschungsanstalt für Viruskrankheiten der Tiere, Insel Riems, Germany*

Transneuronal viral tracing was performed to localize neuroanatomical projections of the trigeminal system which innervates the nasal mucosa of the mouse. Two strains of the neurotropic Pseudorabies virus differing in their glycoprotein expression were applied to the nasal cavity of mice. Viruses replicated in infected neurons and were transported to reach synaptically connected neurons. After different survival times, the animals nasal mucosa, trigeminal ganglia and the whole brains were sectioned and immunostained by antiviral antiserum. Virus-labelled cells appeared in a time dependent fashion in the ganglia and in the ipsilateral brain-stem predominantly at the sensory spinotrigeminal nucleus. The investigated strains showed significant differences concerning timecourse of spread and selectivity towards the trigeminal system.

Viruses could serve as self-amplifying specific markers of connected neurons along hierarchial chains of functionally related circuits. We investigated virus strain-dependent tracing and the detailed topology of trigeminal projections from the periphery to central structures.

65 Poster: Trigeminal Chemoreception & Irritation

ACTIVATION OF BRAIN STEM NEURONS BY IRRITANT CHEMICAL STIMULATION OF THE THROAT ASSESSED BY C-FOS IMMUNOHISTOCHEMISTRY

Cuellar J.M.[1], Boucher Y.[2], Simons C.T.[1], Jung S.[3], Iodi Carstens M.[1], Carstens E.[1] *[1]Neurobiology, Physiology and Behavior, University of California, Davis, Davis, California; [2]Laboratoire de Physiologie de la Manducation, University Paris 7, Paris, France; [3]Anesthesiology, Keimyung University, Daegu, Korea*

We used c-fos immunohistochemistry to identify the brain stem distribution of neurons activated following irritant chemical stimulation of the laryngopharyngeal mucosa. In pentobarbital anesthetized rats, water (control), nicotine (600 mM, 1 ml) or capsaicin (330 μM, 1 ml) was applied to the pharynx by cannula. Following nicotine and capsaicin, there were significant increases in fos-like immunoreactivity (FLI) in: nucleus of the solitary tract from the level of the pyramidal decussation caudally to the level of the area postrema rostrally; dorsomedial aspect of trigeminal subnucleus caudalis (Vc); and paratrigeminal islands interspersed in the spinal trigeminal tract. Capsaicin elicited significantly more FLI in Vc and paratrigeminal nuclei than nicotine, while the reverse held for NTS. There were significant increases in FLI in area postrema and the ventrolateral medullary region dorsal to the lateral reticular nucleus following nicotine but not capsaicin. The distributions of FLI in NTS, area postrema, Vc and paratrigeminal nuclei are consistent with prior anatomical tract-tracing studies, and suggest roles for these brain stem regions in mediating sensory and reflex responses to irritant chemical stimulation of the upper respiratory mucosa. Supported by TRDRP (10DT-0197, 6RT-0231), NIH (DR13685, GM56765) and the International Association for the Study of Pain.

66 Poster: Trigeminal Chemoreception & Irritation

ASSESSMENT OF VARIATION IN NASAL SUBMUCOSAL BLOOD FLOW USING LASER-DOPPLER FLOWMETRY WITH RHINOSTEROMETRY

Konnerth C.[1], Opiekun R.[2], Gould M.[2], Dalton P.[1] *[1]Monell Chemical Senses Center, Philadelphia, PA; [2]Monell Chemical Senses Center, Philadelphia, Pennsylvania*

The use of laser-Doppler flowmetry (LDF) to measure microcirculation in human nasal tissue has shown promise as a way of non-invasively assessing local response to substances that irritate and/or inflame the nasal mucosa. However, repositioning the laser-probe across repeated measurements from the same individual often yields significant variability in the response, due to changes in the depth of the tissue being perfused. Coupling micro-manipulator guided LDF with rhinostereometry (RSM) allows simultaneous measurement of the nasasl bloodflow and mucosal swelling and consistency in the placement of the probe across repeated measurements. We used micro-manipulator guided LDF in combination with RSM to assess blood flow, congestion and temperature in the anterior medial surface of the inferior turbinate in 8 healthy men and women across multiple sessions. A custom-designed, infrared laser-Doppler probe (wavelength: 780 nm) was placed parallel to the mucosal surface, between the anterior-medial part of the inferior turbinate and the cartilaginous part of the nasal septum. A distance of 0.1 to 0.3 mm between the probe tip and the mucosal surface was maintained under direct visual inspection using a micromanipulator. We investigated both the reliability and variability of the baseline response during 10 sessions (5/day). With the addition of special temperature probes to the system, simultaneous correlations between nasal mucosal temperature and blood flow in the anterior turbinate were also evaluated and suggested that under certain challenge conditions, nasal mucosal temperature could be used as a surrogate index for nasal submucosal blood flow. Supported by NIH P50 DC 00214

67 Poster: Trigeminal Chemoreception & Irritation

CHEMESTHESIS FROM ALKALINE DUSTS: DISSOLUTION IN MUCUS AND COURSE OF SENSATION

Cain W.S.[1], Jalowayski A.A.[1], Kleinman M.[2], Schmidt R.[1], Hillen B.K.[1], Magruder K.[1], Lee B.R.[1], Culver B.D.[3] *[1]Chemosensory Perception Lab., Surgery, University of California, San Diego, La Jolla, CA; [2]Particle Inhalation Lab., Commun. & Environ. Medicine, UCI, Irvine, CA; [3]Epidem. Div., Medicine, UCI, Irvine, CA*

A previous study of inhalation of dusts showed that equal molar concentrations of sodium borate (sodium tetraborate pentahydrate) and calcium oxide caused equal chemesthetic magnitude. A time-sensitive dosimetric model built from knowledge of minute ventilation, dust deposited, mucociliary clearance, and nasal secretion shows that at matched molar concentration in air molar concentration dissolving into mucus also matched. This study addresses whether the dosimetric account will hold out to a duration over twice as long as that originally studied. Ten men screened for health of the airways and eyes assessed magnitude in replicate exposures of 45 min to sodium borate at 5 and 9 mg/m3 in air, calcium oxide at 2 mg/m3, and just air. The exposures occurred in a dome that covered the head while.the subject exercised to simulate light work. Other outcome variables included nasal resistance, volume of secretion, and clearance time. For both materials, chemestethic magnitude increased for about a half hour, then declined. The difference in potency seen previously between agents held here as well and in this respect supported the dosimetric model. Nevertheless, the magnitude of the physiological responses indicated that dosimetry probably cannot account for the decline in chemesthetic magnitude over time. A level-dependent adaptation, such as seen in chemesthetic responses to vapors over time, may account for the decline. Supported by U.S. Borax Corp.

68 Poster: Trigeminal Chemoreception & Irritation

EFFECT OF SALIVA COMPOSITION ON TEXTURE PERCEPTION OF MAYONNAISE

Engelen L.[1], Van Den Keybus P.A.[2], De Wijk R.A.[3], Bosman F.[1] *[1]Head and Neck, University Medical Centre Utrecht, Utrecht, Netherlands; [2]Oral Biology, Academic Centre for Dentistry Amsterdam, Amsterdam, the Netherlands; [3]Marketing Research & Sensory Science, ATO, Wageningen, the Netherlands*

During mastication food is mixed with saliva, which among others is essential for bolus formation, initial breakdown and protection of mucosa. Saliva is also involved in our perception of flavor and texture of foods. In order to investigate how the composition of saliva influences the ratings of sensory attributes, components of four types of saliva were correlated with sensory ratings of mayonnaise. Saliva was collected during 5 minutes at rest and after three types of stimulation: odor, parafilm chewing and citric acid (4%). The flow rates were calculated and the saliva was analyzed for various components: buffer capacity, mucin and total protein concentration, and &alpha-amylase activity. Several significant correlations could be found between salivary components and sensory ratings. &alpha-amylase activity was negatively correlated with the sensation of coating and fatty after feels. Protein concentration was also negatively correlated with fatty after feel. Subjects with a higher salivary buffer capacity perceived astringent after feel and heterogeneous mouth feel stronger. These results indicate that the composition of saliva can influence the individual texture ratings of mayonnaise.

69 Poster: Trigeminal Chemoreception & Irritation

ASSESSMENT OF THRESHOLDS FOR TRIGEMINALLY MEDIATED SENSATIONS

Frasnelli J.[1], Hummel T.[1] [1]*ENT-Department, University of Dresden Medical School, Dresden, Germany*

Aim of the study was the investigation of different techniques to assess thresholds for trigeminally mediated sensations following intranasal stimulation. Trigeminal threshold was measured using different techniques. A total of 32 subjects participated; half of the subjects were between 18 and 35 years old (8 female, 8 male subjects), the other half was older than 45 (8 female, 8 male subjects). In all experiments menthol and linalool were used as stimulants: in experiment A, threshold for each odor was determined using an initially ascending staircase method. When subjects indicated the presence of a "trigeminal" sensation three times in a row for a given concentration, the staircase was reversed. The next reversal was triggered when subjects did not indicate the presence of such a sensation, etc. This procedure was continued until 7 reversals had occurred. In experiment B, in a similar design, trigeminal thresholds were estimated by measuring the lateralization threshold, i.e. subjects had to identify which side of the nose had been stimulated following lateralized stimulation. In experiment C, the negative mucosa potential (NMP – an electrophysiologcial measure of trigeminal activation at mucosal level) was measured. All experiments were repeated on different days. Statistical analysis revealed different degrees of test-retest-reliability (all r32>0.41; all $p<0.022$). Differences between groups were only found in the re-test sessions: older subjects were found to have higher thresholds than younger (menthol in experiment B: $p=0.012$). Additionally, women had lower threshold than men (experiment a: all $p< 0.033$). In conclusion, in this study it could be shown that all three techniques allow the estimation of trigeminal thresholds.

70 Poster: Trigeminal Chemoreception & Irritation

QUANTITATIVE AND QUALITATIVE STIMULUS INTERACTIONS AMONG NASAL MUCOSAL IRRITANTS

Shusterman D.J.[1] [1]*Medicine, University of California, San Francisco, San Francisco, CA*

Nasal chemesthesis has traditionally been considered an undifferentiated sensory modality. However, molecular, electrophysiologic, and human psychophysical studies increasingly support a contrary view. The degree of perceptual specificity in trigeminal irritation - as well as interactions between trigeminal irritants with known receptors - was the focus of this pilot study. We applied psychophysical rating of serial stimuli - a technique used extensively in the oral cavity - to the nose. Subjects included 3 males and 3 females, all non-smokers, ranging in age from 29 to 54. On 10 separate days, subjects were presented various permutations of capsaicin (35 ppm), menthol (0.3%), or nicotine (2%), two stimuli per session. Differing (or identical) stimuli, dissolved in a common vehicle, were applied serially to the nasal septum (50 uL in 8 mm dia. filter paper discs) for 5 min. at a time, separated by 10 min. At 2.5 min. intervals, various components of trigeminal sensation (including "tingling," "stinging," "burning," "numbness," and "cooling") were rated using visual analog [labeled magnitude] scales. Menthol showed the most consistent rating pattern (cooling >> all others), followed by nicotine (stinging > burning), then capsaicin (burning > stinging). Menthol showed the most significant evidence of self-desensitization ($p<0.05$ for cooling), followed by nicotine ($p=0.05$ for stinging). Asymetrical cross-desensitization was apparent between capsaicin / nicotine and nicotine / menthol, and cross-*sensitization* was observed when capsaicin followed menthol. If replicated in larger samples of subjects, findings such as these may serve as a basis for hypothesizing signal processing schemata in nasal chemesthesis.

71 Poster: Trigeminal Chemoreception & Irritation

DEVELOPMENT OF AN OCULAR EXPOSURE DEVICE FOR DETECTION OF IRRITATION THRESHOLDS: THE TIDE SYSTEM

Opiekun R.E.[1], McDermott R.[1], Dalton P.[2] [1]*Chemosensory, Monell Chemical Senses Center, Philadelphia, Pennsylvania;* [2]*Chemosensory, Monell Chemical Senses Center, Philadelphia, PA*

Ocular irritation is frequently overlooked as a sentinel biomarker of chemical vapor overexposure. Current occupational exposure standards are primarily based upon respiratory irritation and asphyxia that are brought about by much higher vapor concentrations than those required to initiate adverse ocular symptoms. Since there is a paucity of information on chemical vapor concentrations that would lead to ocular irritation and hyperemia, an instrument has been developed to determine ocular irritation thresholds in human subjects. Results indicate that while ocular irritation thresholds vary widely among individuals, the vapor concentrations required to cause ocular irritation can be substantially lower than those encountered in occupation settings.

72 Poster: Sensory Evaluation & Consumer Research

THE EFFECTS OF ODOR ON WEIGHT PERCEPTION

Allen E.T. Hirsch, A.R., Busse, AM., Hoogeveen J.R.. *Loyola University; Chicago, IL; Smell & Taste Treatment and Research Foundation, Chicago, IL; Hope College, Holland, MI; Dort College, Sioux City, Iowa.*

Our objective was to assess the effects of odorants on men's perception of a woman's weight. According to our methods, a five foot nine inch, 245-pound woman model wore a floral-spice odorant, a citrus-floral odorant, and a sweet pea and lily of the valley odorant. In a double-blinded fashion, one hundred and ninety-nine males assessed the woman's weight. We found that compared to the non-odorant control perceived weight, the floral-spice mixture reduced the men's perception of the woman's weight by 4.1 pounds ($p = 0.02$). In those who viewed this odor hedonically positively, the reduction in weight was 12 pounds ($p = 0.02$). We conclude that wearing a floral-spice odor can reduce a woman's perceived weight by as much as seven percent.

73 Poster: Sensory Evaluation & Consumer Research

TEENAGE ABILITY TO DISCRIMINATE CAFFEINE IN COMMERCIAL SODA

Hirsch A.R.[1], Lu H.H.[2], Ma A.[2] [1]*Neurology and Psychiatry, The Smell & Taste Treatment and Research Foundation, Chicago, Illinois;* [2]*Science, Illinois Mathematics and Science Academy, Aurora, IL*

Ninety-two percent (92%) of adults are unable to detect the difference between caffeinated and decaffeinated cola. We sought to evaluate this ability in teenagers, who comprise a large portion of the caffeinated cola market. Twenty-one (21) high school students, average age 15.8 (range 14-18), eleven male, ten female, who drank an average of 4.4 12-ounce cans per week, underwent a taste test of their preferred cola in the commercially available caffeinated and "caffeine-free" forms. All declared preference for the caffeinated version, 90% (n=19) based this preference on taste. As per Griffith's protocol, each subject underwent 20 force-choice detection tests after a training session. Eighty-six percent (n=18) were unable to detect a flavor difference of more than 14 trials ($p<0.05$). The mean number of correct trials amongst the subjects was 10.57 (53%), not significantly different from chance performance (50%) ($p<0.05$) (1-tailed independent t-test). This discrepancy, between subjects' abilities and perception of the reason for consumption of the preferred cola, highlights a lack of realization as to the motivation for hedonic preferences.

74 Poster: Sensory Evaluation & Consumer Research

EFFECTS OF ODORANT ADMINISTRATION ON OBJECTIVE AND SUBJECTIVE MEASURES OF SLEEP QUALITY, POST-SLEEP MOOD AND ALERTNESS, AND COGNITIVE PERFORMANCE

Raudenbush B.[1], Koon J.[1], Smith J.[1], Zoladz P.[1] [1]*Psychology, Wheeling Jesuit University, Wheeling, West Virginia*

The present study investigated whether odorant administration during sleep affects sleep patterns, mood, cognitive functioning, and alertness. Participants were monitored for 3 nights, during exposure to jasmine odor, lavender odor, and a non-odored condition. Following sleep, participants completed questionnaires related to mood and tests of cognitive functioning, and indicated alertness level throughout the day. Jasmine odor led to greater sleep efficiency and reduced sleep movement, without differences in total sleep time, thus providing increased sleep quality without the need for additional sleep time. Upon awakening, jasmine condition participants rated their level of anxiety and vigor lower, and performed the cognitive tests more rapidly. Level of alertness in the jasmine condition was greater than the control condition during afternoon hours. These findings provide support for odorant administration as an adjunct to improve sleep, alertness, and mental performance. This research was funded by a grant from the National Science Foundation (BCS-0115624) and the National Aeronautics and Space Administration to B. Raudenbush.

75 Poster: Sensory Evaluation & Consumer Research

TREATING USED MUSHROOM "SOIL" TO CONTROL ODORS DURING COMPOSTING

Wysocki C.J.[1], Heinemann P.H.[2], Graves R.E.[2], Beyer D.M.[2], Louie J.[1], Kim J.J.[1], Preti G.[1] [1]*Monell Chemical Senses Center, Philadelphia, Pennsylvania;* [2]*Department of Agricultural and Biological Engineering, Pennsylvania State University, University Park, PA*

Odors generated from agricultural activities can become a nuisance and generate ill will to the point of ligitation. We have been evaluating the possibility of utilizing an aerated, closed system to minimize odors generated during mushroom farming. Spent mushroom substrate was treated (a) in the traditional fashion by turning piles of composting substrate or (b) by placing the material in an aerated enclosure. Odors were evaluated by trained panelists in the field or were collected for chemical analysis by GC and GC/MS and additional evaluation in the laboratory by different trained sniffers. Results indicated that aeration significantly reduced odor production whether evaluated in the field or in the laboratory (for samples taken directly from the sources, there was good agreement between field and laboratory panelists); reduced volitile sulfur compounds and other VOCs; and may have shortened the time needed to complete the process.

76 Slide: Human Psychophysics: Taste and Trigeminal

INHIBITION OF SWEET TASTE BY ZINC

Keast R.S.[1], Canty T.M.[1], Breslin P.A.[1] [1]*Monell Chemical Senses Center, Philadelphia, PA*

There are many structurally divergent stimuli that elicit sweetness including the ions of lead and beryllium. There are no known ionic inhibitors of sweetness in humans. We show here that the metal ion zinc is a potent inhibitor of sweetness. The present study investigated the influence of zinc on perceived sweetness of several sweeteners. Subjects (n=15) were screened for their ability to identify basic taste qualities and astringency, and trained in the use of the general Labeled Magnitude Scale. Nine diverse sweeteners (Glucose, Fructose, Aspartame, Saccharin, Acesulfame-K, Sucralose, Sorbitol, Thaumatin, and Neohesperidin dihydrochalcone) were matched for sweetness intensity to 300 mM sucrose. Five salt additives (25 mM) were used: sodium acetate, sodium salicylate, magnesium sulfate, magnesium acetate, and zinc acetate. All possible binary combinations of ten sweeteners (including sucrose) and five salts were tested. Of the salts tested, only zinc acetate significantly inhibited perceived sweetness ($p<0.05$). Zinc acetate inhibited >80% of the sweetness of most compounds tested. There was no significant difference in the magnitude of sweetness inhibition by zinc among the compounds. These findings suggest that these sweeteners either act on a common receptor, multiple receptors with a common component (e.g., T1R2), or a small class of closely related receptors that are inhibited by zinc. We will also discuss the influence of zinc on salty, savory, sour, and bitter tasting stimuli for comparison. Supported by NIHDC 02995 to PASB.

77 Slide: Human Psychophysics: Taste and Trigeminal

IMMEDIATE AND DELAYED TASTE CONTRAST IN YOUNGER AND OLDER ADULTS

Specht S.M.[1], Alberico K.F.[1], Maher J.D.[1], Mastrangelo L.E.[1] [1]*Psychology, Utica College, Utica, NY*

In the gustatory system, response to a target stimulus will differ depending upon the stimuli which precede it. This phenomenon is known as successive taste contrast (e.g., Rankin and Marks, 1991; Schifferstein and Frijters, 1992; Specht & Twining, 1999). Given the fact that sensory abilities change with normal aging (see Schiffman, 1997) and that older adults have alterations in gustatory thresholds (see Mojet, Christ-Hazelhof & Heidema, 2001) and decreased abilities in chemosensory indentification (e.g., Stevens & Cain, 1987), it is potentially clinically relevant to investigate taste contrast in older adults. Younger participants (mean age = 20.4 yrs) and older participants (mean age = 76.1 yrs.; recruited from programs within the Oneida Co. Office for the Aging) were randomly assigned to groups of a 2 (Sequence) X 2 (Interval) X 2 (Age) factorial design. Each participant was randomly assigned to taste either, a 4% sucrose solution followed by another 4% sucrose solution or a 32% sucrose solution followed by a 4% sucrose solution. Participants rated "sweetness" of the solutions on a visual analog scale. Participants rated the second solution either immediately following, or one week after the first solution (i.e., a one week ISI). A 2 (Sequence) X 2 (Interval) X 2 (Age) ANOVA with subsequent post hoc analyses revealed that successive taste contrast was evident (participants rated 4% sucrose as less "sweet" when it was preceded by 32% sucrose) for both younger and older participants and that taste contrast occurred even after a one-week ISI.

78 Slide: Human Psychophysics: Taste and Trigeminal

TEMPORAL PROCESSING OF TASTE MIXTURES LIMITS THE IDENTIFICATION AND ORDER OF PERCEPTION OF COMPONENTS

Laing D.G.[1], Marshall K.[1], Jinks A.[1], Effendy J.[1], Hutchinson I.[1]
[1]Centre For Advanced Food Research, University of Western Sydney, Richmond, Australia

The capacity of humans to identify the components of taste mixtures is limited to 3 (Laing et al 2002). Here the hypothesis that temporal processing differences ie differences in onset times, have a major role in limiting capacity is investigated. 32 subjects were trained to identify sucrose, sodium chloride and citric acid at 6 concentrations and indicate 1) which tastant was perceived first and 2) the identity of each component in binary and ternary mixtures. Concentrations of components were adjusted to provide some intensity conditions where time differences in processing the individual components were minimized. With Binary Mixtures systematic changes in intensity resulted in identification of only 1 component when the differences were largest, and both when intensities were similar. In contrast, subjects found it difficult to indicate which component was perceived first when the intensities were similar and easy when substantially different. More profound effects were found with Ternary Mixtures. With 8/18 mixtures, subjects could not indicate which component was perceived first, and in 15/18 mixtures not all components were identified above chance. Indeed in 18/18 mixtures the most common number of components correctly identified was 2. The results support the hypothesis that temporal processing and working memory play a major role in limiting the identification of tastants in mixtures, and demonstrate that the gustatory system has similar restrictions on its capacity to process information from mixtures as the olfactory system.

79 Slide: Human Psychophysics: Taste and Trigeminal

DEGREE OF DOSE ADDITION IN CHEMOSENSORY DETECTABILITY OF MIXTURES: A WINDOW INTO THE BREADTH OF CHEMICAL TUNING IN CHEMORECEPTION

Cometto-Muniz J.E.[1], Cain W.S.[1], Abraham M.H.[2] *[1]Surgery (Otolaryngology), University of California, San Diego, La Jolla, California; [2]Chemistry, University College London, London, United Kingdom*

We continue to explore the extent of dose addition, and the role that structure and properties might play, in the olfactory and trigeminal detectability of binary mixtures of airborne chemicals by humans. We will present data on the compounds ethyl propanoate and ethyl heptanoate, and analyze it in the context of the outcome from the previous pairs 1-butanol/2-heptanone and butyl acetate/toluene. Our strategy consists in measuring concentration-detection (i.e., psychometric or detectability) functions for the single substances and then, based on this information, preparing binary mixtures where the components vary in their proportions but, if a rule of dose addition were to hold, the selected mixtures should be equally detectable. Detectability is measured via a three-alternative forced-choice procedure and all stimuli are quantified in the vapor phase by gas chromatography. Results obtained so far indicate that the degree of dose addition for both olfaction and chemesthesis is quite complete at relatively low levels of detectability but decreases as the detectability of the mixtures approaches perfect detection. The decrease in dose addition with increased detectability is much more pronounced in olfaction than in chemesthesis. The outcome leads us to conclude that chemical tuning is narrower in olfaction than in either nasal or ocular chemesthesis. Supported by grant number R01 DC 02741 from the NIDCD, NIH.

80 Slide: Human Psychophysics: Taste and Trigeminal

TIME-INTENSITY RATINGS OF NASAL IRRITATION FROM CARBON DIOXIDE

Wise P.[1], Radil T.[2], Wysocki C.J.[2] *[1]Monell Chemical Senses Center, Philadelphia, Pennsylvania; [2]Monell Chemical Senses Center, Philadelphia, PA*

Studies examined time-intensity profiles of nasal irritation from carbon dioxide (CO_2). A Kobal-type olfactometer presented dilutions of CO_2 (0 to 70%) for up to ten seconds. Subjects continuously tracked intensity of nasal sensation (using a labeled magnitude scale) by moving a slider. Study 1 showed that, on average, rated intensity peaked about 3-4 seconds after stimulus-onset and fell rapidly thereafter. However, large individual differences occurred (some subjects reported complete cessation of sensation before stimulus-offset, whereas others reported little or no decrease). These differences proved stable across sessions. Study 2 suggested that a) temporal integration (for a high concentration of CO_2) was complete for a 2-second stimulus and b) perceived intensity decreased more rapidly for 2-second than for 7-second stimuli. Control studies suggested that individual differences in ability to maintain velopharyngeal closure (Studies 3 and 5) and in tendency to rate dryness (low humidity) as nasal irritation (Study 4) fail to account for individual differences in time-intensity profiles. Study 6 suggested that the nose rapidly regains sensitivity with very brief (300-500 millisecond) interruptions in presentation of CO_2. In summary, on average, the nose seems to desensitize rapidly to CO_2 and recover rapidly with gaps in stimulation. However, large individual differences in the rate of desensitization occur. Future studies may determine the factors underlying the temporally dynamic response to CO_2 and causes of individual differences.

81 Symposium: Hanging by a Thread: Scaffolds in Signal Transduction

TARGETING AND SCAFFOLDING OF EPITHELIAL RECEPTORS AND ION CHANNELS

Milgram S.L.[1] *[1]Cell and Developmental Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*

Epithelial cells play important roles in host cell defense, nutrient absorption, and ion transport. It is well known that many hormones modulate epithelial cell function by acting on basolateral receptors but epithelia are also regulated by signals originating from the apical surface. We are interested in identifying mechanisms that target receptors to the apical membrane and in defining protein interactions that coordinate signaling from these receptors. Using site-directed mutagenesis we identified a COOH-terminal apical targeting sequence in the intestinal heat-stable enterotoxin receptor guanylyl cyclase C (GCC). Using yeast two hybrid and proteomic approaches we find that GCC associates with apical membrane PDZ proteins, but this interaction is not required for efficient targeting or retention of GCC at the plasma membrane. Rather the interaction with epithelial PDZ proteins can modulate signaling via GCC and coordinates a multiprotein scaffold that links GCC and apical ion channels and transporters, including the cystic fibrosis transmembrane conductance regular (CFTR) Cl- channel. We find similar signaling scaffolds involved in regulating the activity of CFTR via receptors that activate the cAMP - PKA signaling pathway and are using proteomic approaches to define the composition of these protein complexes. Supported by NIH grants and the Cystic Fibrosis Foundation.

82 Symposium: Hanging by a Thread: Scaffolds in Signal Transduction

A CHOLESTEROL REGULATED PP2A/HEPTP COMPLEX WITH DUAL SPECIFIC ERK1/2 PHOSPHATASE ACTIVITY

Anderson R.G.[1] [1]*Cell Biology and Neuroscience, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas*

Cholesterol is a structural element of the lipid scaffold that regulates signal transduction from caveolae/rafts. Previously we have shown that the acute depletion of membrane cholesterol causes the concentration of pERK1/2 in caveola/raft lipid domains and the cytosol of human fibroblasts to dramatically increase. This increase could be caused either by the activation of MEK-1 or the inhibition of a pERK phosphatase. We have isolated a high molecular weight (~440 kDa), cholesterol regulated pERK phosphatase that dephosphorylates both the phosphotyrosine and the phosphothreonine residues in the activation loop of the enzyme. The dual activity in the complex appears to be due to the combined activities of the serine/threonine phosphatase PP2A and the tyrosine phosphatase HePTP. Acute depletion of caveolae cholesterol causes the disassembly of the complex and a concomitant loss of the dual specific pERK phosphatase activity. The existence of a cholesterol regulated HePTP/PP2A activity provides a molecular explanation for why ERK activity in caveolae is sensitive to membrane cholesterol levels and raises the possibility that ERK plays a role in regulating the traffic of cholesterol to caveolae/rafts and other membranes.

83 Symposium: Hanging by a Thread: Scaffolds in Signal Transduction

MOLECULAR SCAFFOLDS AND INTERACTING PROTEINS AFFECT ION CHANNEL FUNCTION IN THE OLFACTORY SYSTEM

Fadool D.A.[1] [1]*Prog. in Neurosci. & Mol. Biophysics, Florida State University, Tallahassee, Florida*

Protein-protein interactions are a mechanism to place proteins such as ion channels in close proximity to interfacing signal transduction elements that regulate their electrical activity. Proteins may contain domains (SH2, SH3, PDZ, WW) that target conserved recognition motifs in an interacting protein to bind the partner proteins in a phosphorylation-dependent or -independent manner. This symposium talk will highlight common examples of scaffolds in the mammalian nervous system using tyrosine kinase signaling pathways in the olfactory bulb (OB) as a model. Multiple tyrosine kinases modulate a voltage-gated potassium channel (Kv1.3) expressed in the OB neurons through site-specific tyrosine phosphorylation of the channel. Adapter proteins contained in the neurotrophin, src kinase, and insulin signaling pathways alter the phosphorylation, functional biophysics, expression, and membrane trafficking of the channel by assembling different multicomponent complexes. The nature of the complex can be affected by developmental state or neuronal activity and is left unbalanced when the ion channel is removed by gene-targeted deletion. In summary, binary protein-protein interactions are the heart of signal transduction, however, multicomponent complexes dominate neuronal signaling and the role of scaffolds and adaptor molecules should be considered when studying sensory transduction in the olfactory and taste systems. Supported by NIH R01DC03387.

84 Poster: Chemical Ecology

ESCAPIN: AN ANTIPREDATOR PROTEIN IN THE DEFENSIVE SECRETION OF *APLYSIA CALIFORNICA*

Johnson P.M., Yang H., Tai P.C., Derby C.D. *Biology, Georgia State University, Atlanta, GA*

Many organisms produce chemical defenses to deter predation, yet the behavioral, neurophysiological, and cellular mechanisms of chemical defenses are largely unexplored. The seahare *Aplysia californica* releases defensive secretions called ink and opaline from independent glands. In behavioral assays, seahares with full glands versus those with depleted glands had a significant survival advantage against their primary predator, the great green sea anemone *Anthopleura xanthogrammica*. Isolated secretions and their components were used to explore mechanisms of defense against sea anemones. Ink was found to contain a dominant 60 kDa glycoprotein ("escapin"), which was then isolated, cloned, sequenced, and expressed in *E. coli*. Escapin was sufficient to generate aversive reactions of sea anemones, perhaps related to its ability to lyse anemone cells. To examine whether escapin is necessary for ink's deterrent effects, RNA interference experiments are being conducted. Double stranded RNA was generated from the cloned escapin gene and injected into the ink glands of adult *A. californica*. Translation of escapin was inhibited, as verified by SDS-PAGE. We are currently conducting behavioral experiments with dsRNA-injected and sham-injected animals to determine if escapin is necessary for survival of encounters with sea anemones. Escapin is the first reported antipredator protein of any organism. Supported by NSF IBN-0077474 and the NSF Center for Behavioral Neuroscience (IBN-9876754).

85 Poster: Chemical Ecology

FEEDING RESPONSES TO SELECTED TREE SPECIES AND PHYTOCHEMICALS BY GYPSY MOTH LARVAE, *LYMANTRIA DISPAR* (L.)

Shields V.D.[1], Broomell B.P.[1], Rodgers E.J.[1], Salako J.O.[1], Dodson L.K.[1], Agbasi K.T.[1] [1]*Biological Sciences, Towson University, Towson, Maryland*

Insects depend largely on their sense of taste and smell to find food. In lepidopterous larvae, feeding is controlled by input from gustatory chemosensilla located on the mouthparts. When a caterpillar bites into a leaf, these sensilla detect phytochemicals present in the plant. This taste information is coded by receptor cells in these sensilla and sent to the brain of the insect. The neural message determines whether the insect eats or rejects the food. The larva of the gypsy moth *Lymantria dispar* L. is considered to be one of the most serious forest pests of North America. To better understand the feeding behavior of this polyphagous insect, we examined the feeding preferences of fifth instar larvae using a two-choice leaf disc assay. Seven overstory tree species were selected from the Towson University campus. These included: sweet gum, *Liquidambar styraciflua*, sugar maple, *Acer saccharum*, tulip poplar, *Liriodendron tulipifera*, American beech, *Fagus grandifolia*, American basswood, *Tilia americana*, red oak, *Quercus rubra*, and black walnut, *Juglans nigra*. Our results revealed a clear hierarchical order of feeding preference. In addition, using a similar experimental design, we tested the feeding responses of these larvae to selected phytochemicals when each was applied to natural (leaf) versus artificial (filter paper) disks. We found that many of the phytochemicals tested were deterrent and only a few were stimulatory. The remainder of the compounds was neutral. Supported by NIH 3 R25 GM58384-01A2S1 and NSF DBI-0097478 grants and Towson University.

86 Poster: Chemical Ecology

CO_2 EMISSION BY DATURA FLOWERS AND ITS SIGNIFICANCE FOR FORAGING *MANDUCA SEXTA* MOTHS

Thom C.[1], Guerenstein P.G.[1], Yepez E.[2], Mechaber W.L.[1], Vanharen J.[3], Hildebrand J.G.[1], Williams D.[2] [1]*ARL Division Of Neurobiology, University of Arizona, Tucson, AZ;* [2]*School of Renewable Natural Resources, University of Arizona, Tucson, AZ;* [3]*Biosphere-2 Center, Oracle, AZ*

The nocturnal hawkmoth *Manduca sexta* feeds on nectar of *Datura wrightii* (Solanaceae) flowers, which produce nectar during the night. As nectar production requires high metabolic activity, a high floral CO_2 emission may signal food abundance to the moths. Adult *M. sexta* possess a highly developed CO_2-perception system. However, the role of CO_2 information in the biology of this and other Lepidoptera is largely unknown. We asked if the moths could use floral CO_2 emission to identify a food source. Using a state-of-the-art InfraRed Gas Analyzer, we measured CO_2 release from *D. wrightii* flowers in the field, as well as nectar production. Our results suggest that (1) early at night, *D. wrightii* flowers produce CO_2 levels that are well above the background and that could be detected by the moths, and (2) production of both CO_2 and nectar decreases significantly during the night. We also tested the behavioral response of the moths to flowers emitting different CO_2 flows. Choice experiments were done in a flight tent with artificial flowers attached to natural hostplants. Supported by the German Academic Exchange Service, and grants NIH R01-DC-02751 and NSF IBN 9983302

87 Poster: Chemical Ecology

LIFE STAGE AND ODORANT-INDUCED CHANGES IN OLFACTORY SENSITIVITY IN COHO SALMON, *ONCORHYNCHUS KISUTCH*

Dittman A.[1], Baldwin D.[1], May D.[1], Scholz N.[1] [1]*Northwest Fisheries Science Center, Seattle, WA*

Over the lifetime of an organism, the sensitivity of the olfactory system to specific odors may change in response to developmental changes, hormones, environmental stimuli, and odorant exposure. Salmon provide an excellent model for studying such changes because almost every aspect of their lives is influenced by olfaction and they experience dramatic developmental and environmental transitions (smolting, maturation, freshwater vs. oceanic) as part of their migrations from freshwater to oceanic feeding grounds and back. Furthermore, these homing migrations are governed by olfactory discrimination of home stream odors that juvenile salmon learn (imprint to) prior to their seaward migrations. Our previous studies demonstrated that salmon imprinted to the odorant phenylethyl alcohol (PEA) developed a long-term sensitization of peripheral olfactory neurons to this odorant. To further examine this imprinting phenomenon, we exposed juvenile coho salmon, *Oncorhynchus kisutch* to three distinct classes of odorants (amino acids, bile acids and PEA) during smolting, the presumptive sensitive period for imprinting. To assess life stage and olfactory imprinting associated changes in the sensitivity of the olfactory system, we recorded electrical field potentials (electro-olfactograms) generated in response to these three classes of odorants and ovarian fluid at four distinct life stages: oceanic juvenile, maturing adult , immature adult, and mature. Our results suggest that olfactory sensitivity changes over the lifetime of the salmon and previous odor exposure can influence olfactory responses. Funded by BPA Grant #199305600

88 Poster: Chemical Ecology

CARBOHYDRATE SENSITIVITY OF CRAYFISH (*PROCAMBARUS CLARKII*) LEG CHEMORECEPTOR CELLS

Corotto F.S.[1], Fant M.J.[1] [1]*Department of Biology, North Georgia College and State University, Dahlonega, GA*

Suction electrode techniques were used to examine carbohydrate sensitivity of *P. clarkii* leg chemoreceptors. Chemosensory axon bundles were identified by responses to fish food extract (TET) or high KCl - 90% of the bundles responded to both. We tested six monosaccharides, three carboxylic acid derivatives, three alcohol derivatives, and five amino derivatives of monosaccharides as well as the oligosaccharide stachyose and the disaccharides isomaltose and trehalose. All were tested at ~100 μM on at least 20 chemosensory bundles. The only compound to elicit responses was trehalose, a known stimulus included here to allow comparison with earlier results. We also encountered some KCl-sensitive, TET-insensitive cells with large, mechanoreceptor-like spikes. As bimodal chemo-/mechanoreceptor cells have been identified in other crayfish (Hatt, J. Comp. Physiol. A, 159:611-617), we tested seven KCl-sensitive bundles with TET, pyridine, pyrazinamide, nicotinamide, and a mixture of the seven known chemical stimuli identified previously (Corotto and O'Brien, J. Chem. Ecol., 28:1117-1130). No bundles responded to pyridine, pyrazinamide, or nicotinamide. Some putative mechanoreceptors with large spikes responded only to KCl. Evidence for bimodal cells was not obtained. In summary, previous work has demonstrated sensitivity of *P. clarkii* pereopods to several disaccharides, but our results here show that monosaccharides and their derivatives are not effective at the concentration tested. Initial results also suggest that *P. clarkii* lacks the bimodal chemo-/mechanosensory cells and pyridine sensitivity found in other crayfish.

89 Poster: Chemical Ecology

GROWING CRAYFISH ON DIFFERENT DIETS ALTERS SUBSEQUENT FORAGING DECISIONS: THE IMPACT OF DETRITUS AND FISH DIETS ON CHEMOSENSORY DECISIONS

Adams J.A.[1], Tuchman N.C.[2], Moore P.A.[1] [1]*Biological Sciences, Bowling Green State University, Bowling Green, Ohio;* [2]*Biology, Loyola University of Chicago, Chicago, Illinois*

Chemoreception mediates foraging decisions in many animals and may be affected by past feeding experience. We tested whether the selection of detritus was affected by previous diet experience in crayfish. Detrital leaves from trembling aspen were grown under elevated CO_2 conditions, which alter both the nutritional quality and secondary defensive chemistry of leaf litter. Crayfish were raised from hatch for ten months on three different diets: 1) ambient CO_2-produced detritus, 2) elevated CO_2-produced detritus, or 3) fish. A Y-maze was used to test for preference between stimuli produced from either ambient or elevated CO_2-produced detritus. Crayfish were offered three types of stimuli: 1) fresh detritus, 2) detritus that had been leached in water for 24 hours, and 3) leachate from detritus. Within each stimulus type and for each dietary condition, pair-wise combinations of stimuli (ambient CO_2, elevated CO_2, and a no stimulus control) were tested for crayfish preference. Behavioral parameters quantified included initial arm choice, proportion of time spent in each arm, and proportion of time spent at each stimulus source. Crayfish that had been raised on either ambient or elevated CO_2 detritus showed a preference for ambient CO_2 detritus when paired with either control or elevated CO_2 detritus stimuli. There was no response to detrital stimuli by crayfish that had been raised on a fish diet. Therefore, previous exposure to detritus seems to be important in perceiving detritus as a food source, but not in the preference exhibited by these animals.

90 Poster: Chemical Ecology

THE EFFECTS OF THE HERBICIDE METOLACHLOR ON AGNOSTIC BEHAVIOR IN THE CRAYFISH *ORCONECTES RUSTICUS*

Cook M.E.[1], Moore P.A.[1] [1]*Biological Sciences, Bowling Green State University, Bowling Green, Ohio*

Previous research suggests that agricultural herbicides interfere with olfactory-mediated behaviors such as responses to alarm signals and the ability to locate food. Crayfish use chemical signals during aggressive interactions and these signals are important in establishing dominance, which can impact an individual's ability to find mates, food, and habitat space. In this study, we investigated whether exposure to sub-lethal levels of the herbicide metolachlor would influence the ability of crayfish to respond to olfactory signals used in agnostic behaviors. Crayfish were exposed to four different environmentally relevant concentrations of metolachlor for 96 hours. Each exposed animal was then placed in a fight arena and were allowed to interact with a naïve individual for 20 minutes. Detailed analysis of fight dynamics show that metolachlor does impair the ability of crayfish to receive and respond to olfactory signals used in agonistic encounters.

91 Poster: Chemical Ecology

THE SCENT OF SPECIATION: PERIPHERAL CHEMORECEPTION IN THE *RHAGOLETIS* SPECIES COMPLEX

Olsson S.B.[1], Linn, Jr. C.E.[2], Roelofs W.L.[2] [1]*Neurobiology and Behavior, Cornell University, Ithaca, NY;* [2]*Entomology, NYS Agricultural Experiment Station, Cornell University, Geneva, NY*

The recent shift of *Rhagoletis pomonella* (a true fruit fly) from its native host hawthorn to introduced, domestic apple has been implicated as an example of incipient sympatric speciation. Ecological, behavioral, and genetic studies indicate that differences in host plant preference are important in reproductively isolating *pomonella* flies via premating barriers to gene flow. Single cell electrophysiology was used to determine if differences in peripheral chemoreception could contribute to host preference and fidelity in the *pomonella* group. Individuals from apple, hawthorn, and flowering dogwood origin populations (n=29) were analyzed as well as *R. mendax*, the most closely related confirmed species (n=8). Fourteen volatiles were selected as stimuli from electroantennographic and/or behavioral studies of host fruit. Cluster analysis of 101 olfactory receptor neuron (ORN) responses revealed similar chemical selectivity to the host volatiles (i.e. ORN types were similar in all populations). However, population comparisons revealed significant differences in ORN sensitivity and adaptation time (Kruskal-Wallis H $p<0.001$, 0.006). It is concluded that differences in peripheral sensitivity and adaptation may contribute to behavioral discrimination of host fruit and influence host fidelity in the *pomonella* group. Furthermore, this study reveals the only current morphological or physiological criteria by which *R. pomonella* host races can be distinguished. This research is funded by the Paul J. Chapman Award and NSF Grant #DEB-9977011.

92 Poster: Chemical Ecology

HUMAN SKIN ODORS

Preti G.[1], Bazemore R.[2], Leyden J.J.[3], Foglia A.[3], Smith J.M.[2], Spielman A.I.[4] [1]*Monell Chemical Senses Center, Philadelphia, PA;* [2]*Monell Chemical Senses Center, Philadelphia, PA;* [3]*Dermatology, University of Pennsylvania, Philadelphia, PA;* [4]*College of Dentistry, Basic Science Division, New York University, New York, NY*

Human skin odors result, in part, from interactions of sebaceous and eccrine gland secretions with the resident cutaneous microflora; in vitro, these organisms generate small, volatile odor molecules when incubated with skin secretions. Sebaceous glands are androgen dependent, as evidenced by their growth and abundance of sebum occurring at puberty. Conversely, skin secretions become less abundant in elderly individuals. These changes effect skin microflora which proliferate with moisture and high sebum levels. Therefore, skin odor should change with age and gender and may be indicators of neoplastic changes. Studies have examined skin odors for mosquito attractants and their effect upon fragrances but have generally employed limited numbers of young volunteers. We are studying skin odors in subjects of different age and ethnic groups. Our initial studies in this area have used Solid Phase Microextraction (SPME) and solvent extraction to examine skin volatiles from males of different ethnicity and age. Skin microflora and proteins were also examined on skin adjacent to extracted areas. Cutaneous microflora were similar for all individuals, regardless of age; *Staphylococcus epidermidis* was the dominant organism. Hair may elevate the presence of other organisms. SPME-collected volatiles were analyzed by GC/MS and appear to be qualitatively similar for all individuals examined. Although n-C_9 and C_{10} aldehydes were present, we saw no evidence for the previously identified 2-nonenal, alleged to be an odor linked to "old men." Young males generally appeared to have less volatiles than some older males. Extracts contained high concentrations of C_8-C_{18} acids, and n-C_{12}, -C_{14} and –C_{16} alcohols. Supported by NIH (DC 01072 and T32 DC00014).

94 Poster: Olfactory CNS Processing

DENDRITIC CALCIUM PLATEAU POTENTIAL IN JUXTAGLOMERULAR CELLS IN THE RAT OLFACTORY BULB

Zhou Z.[1], Xong W.[1], Chen W.R.[1], Hines M.L.[2], Shepherd G.M.[1]
[1]Neurobiology, Yale University, New Haven, Connecticut; [2]Computer Science, Yale University, New Haven, Connecticut

The olfactory glomerulus is a structural and functional unit for olfactory information processing. There are three types of juxtaglomerular (JG) neurons around the glomerulus: periglomerular (PG) cells, external tufted (ET) cells and short axon (SA) cells. Both *in vivo* and *in vitro* studies have reported that some PG and ET cells have a long-lasting plateau potential following sodium spikes. We investigated the mechanism for the generation of the plateau potential. The presence of a threshold suggested a Na^+, Ca^{2+} and/or NMDA spike-like mechanism. APV(-) (50 μM) had no effect, indicating that the plateau potential is not due to an NMDA spike. TTX perfusion could not block the plateau potential whereas perfusion of 250-500 μM Ni^{2+} greatly inhibited it, which indicated the involvement of low-threshold Ca^{2+} channels. 500 μM TTX and 50 mM Ni^{2+} puffed near the soma blocked only Na^+ spikes leaving the plateau potential intact, suggesting that the origin of the plateau potential is in the dendrites at a distance from the soma. This result also showed that Na^+ spikes are initiated near the soma in PG and ET cells. Using two-photon microscopy, we studied the Ca^{2+} signal in the soma and dendrites. Single or a few Na^+ spikes could not evoke a Ca^{2+} signal in the dendrites; the Ca^{2+} signal was generally correlated with a plateau potential, during which it continued to increase. Our results suggest that the plateau potential recorded in the soma may reflect passive spread of temporally and spatially summated dendritic Ca^{2+} spikes. Supported by NIDCD, Human Brain Project, MURI grant and 1 PO1 DC 04732

95 Poster: Olfactory CNS Processing

SPONTANEOUS ACTIVITY OF MITRAL CELLS IN THE RAT AND MOUSE

Nica R.[1], Mast T.G.[1], Griff E.R.[1] *[1]Biological Sciences, University of Cincinnati, Cincinnati, OH*

We examined the spontaneous activity of mitral cells and analyzed the mean rates and interspike interval histograms (INTHs) to determine whether subclasses of mitral cells could be identified. Mitral cells, identified by antidromic activation from the posterior piriform cortex, collision tests, and dye markings of the recording sites in the main olfactory bulb, were recorded extracellularly in vivo for at least 600 s from rats and mice anesthetized with chloral hydrate. Under these conditions, most mitral cells exhibited a mean spontaneous activity between 11 and 19 spikes/sec; some cells had mean rates above 20 spikes/sec; a few had mean rates less than 10 spikes/sec. In some cells, spontaneous activity slowly oscillated with the mean rate changing as much as 100% over several minutes. The INTHs of most mitral cells were dominated by bursts of action potentials between 125 and 250 Hz separated by longer intervals; other cells lacked these higher frequency bursts. These two patterns of INTHs were observed across the full range of spontaneous activity mean rates. Thus, bursting cells and non-bursting cells were found with low, medium, and high mean rates. Furthermore, in cells where activity oscillated, the pattern of interspike intervals during the phase when the cell fired faster was similar to the pattern when the cell fired more slowly. Thus, interspike intervals may be an important feature of mitral cells, and there may be two subclasses of mitral cells based on the pattern of interspike intervals in the spontaneous activity. This work was supported in part by NIH grant 1R15DC04548 to ERG and a Sigma Xi GIAR to TGM.

96 Poster: Olfactory CNS Processing

PRE- AND POST-SYNAPTIC PATTERNS OF BULBAR ACTIVATION FOLLOWING ODOR STIMULATION OF THE ZEBRAFISH OLFACTORY EPITHELIUM

Deng P.[1], Bryner B.[1], Michel W.C.[1] *[1]Physiology, University of Utah SOM, Salt Lake City, UT*

The goal of our experiment was to determine the regional distribution of odor-stimulated pre-synaptic and post-synaptic activity in the zebrafish olfactory bulb (OB). We stimulated the OB with agmatine (AGB), a potent odorant in zebrafish and a cationic channel permeant probe. Two experiments were conducted. In the first experiment, we stimulated the olfactory rosettes in vivo with 5 mM AGB for 2h and examined the distribution of AGB immunoreactivity (IR) in the olfactory epithelium and OB. In the second experiment, the olfactory epithelium and bulb of decapitated fish were both exposed to 5 mM AGB for 20 minutes to label both pre-synaptic fibers and active mitral cells by AGB permeation through ionotropic glutamate receptors (Edwards & Michel, J.Comp. Neurol. 2002). Fixed tissue was embedded in resin and semithin sections were collected for immunocytochemistry. Digital images of consecutive sections, stained for AGB, glutamate, taurine and GABA IR, were registered and analyzed using Photoshop 5.5, PCI Geomatica and Image-Pro Plus 4.0 software. Following long-term AGB stimulation of the olfactory epithelium, AGB labeled olfactory receptor nerve (ORN) axons were consistently seen in the ventrolateral area of the entire OB. In the second experiment labeled mitral cells were detected in the ventrolateral olfactory bulb. Our study provides evidence that AGB selectively activates ORNs terminating in the ventrolateral region of the zebrafish olfactory bulb. These ORNs are synaptically coupled to mitral cells primarily located in the same region. Supported by NIH grants DC-01418 and NS-07938.

97 Poster: Olfactory CNS Processing

LOW-THRESHOLD CALCIUM SPIKES IN TURTLE OLFACTORY BULB GRANULE CELLS

Pinato G.[1], Midtgaard J.[1] *[1]Dept. Medical Physiol., Univ. of Copenhagen, Copenhagen, Denmark*

Granule cells are making reciprocal synapses with mitral cell secondary dendrites, thereby controlling the output of the olfactory bulb. In order to analyze the electrophysiological properties of granule cells, current-clamp whole-cell recordings were obtained in the adult turtle olfactory bulb in vitro at physiological temperature. In normal medium two types of spikes were readily observed: fast, TTX-sensitive Na spikes, and a slow LTS, evoked subthreshold for the Na spikes. The LTS was sensitive to low concentrations of NiCl2 and mibefradil, consistent with a T-channel mediated low-threshold calcium spike. 4AP or TEA had minimal effects on the LTS while high-threshold calcium spikes were evoked following potassium channel blockade. The HTS was relatively insensitive to NiCl2, but was blocked by CdCl2. In normal medium, the LTS could in some cases be evoked unaccompanied by Na spikes; in other cases the LTS was crowned by a Na spike. Repetitive stimulation resulted in a gradually diminished LTS and showed a refractory period of a few seconds for the production of the full-amplitude LTS. The refractoriness was reduced by hyperpolarizing interstimulus membrane potentials. Similarly, the LTS-evoked Na spiking was reduced during repetitive stimulation on a timescale comparative to the LTS′ refractory period. The results show that olfactory granule cells possess a strong LTS, which contribute to the excitability sub- and supra-threshold for the Na spikes on a timescale of seconds. Supported by the European Union, the Carlsberg Foundation and the Lundbeck Foundation.

98 Poster: Olfactory CNS Processing

SEXUALLY DIMORPHIC AND ISOMORPHIC FEATURES EXHIBITED BY SOME MAIN OLFACTORY BULB GLOMERULI

Schaefer M.L.[1], Oliva A.M.[2], Restrepo D.[2] [1]*Howard Hughes Medical Institute, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD;* [2]*Department of Cellular and Structural Biology and Neuroscience Program, University of Colorado Health Sciences Center, Denver, CO*

Molecularly defined glomeruli, P2 , of juvenile and adult male and female mice were compared in order to investigate the nature and extent of sexual dimorphism of the first central processing center of this muridae species. In 5-1/2 wk old mice, homologous P2 glomeruli do not differ in volumetric size between males and females. In contrast, homologous P2 glomeruli of 12 wk old mice are sexually dimorphic. Interestingly, the homologous lateral P2 glomeruli differ in size while the medial do not. The lateral P2 glomeruli in females are larger. To begin to investigate whether this is P2-specific or a local phenomenon within the MOB, glomerular cross-sectional areas were measured throughout the bulbs of both genders. Glomeruli residing within the lateral but not the medial domain of the P2 glomerulus, defined as the 95% CI for the mean position of P2, exhibit larger areas in females. These results indicate that glomeruli formed from the same olfactory receptor neuron types exhibit both sexually dimorphic and isomorphic features dependent on their topographic location in the main olfactory bulb. Supported by grants from the NIMH (MH12438) to M.L.S. and NIDCD (DC00566, DC00244, and DC004657) to D.R

99 Poster: Olfactory CNS Processing

RHINOTOPY IN THE LARVAL STAGE OF THE SEA LAMPREY, PETROMYZON MARINUS,

Zielinski B.S.[1], Arbuckle W.J.[1], Firby A.E.[1] [1]*Biological Sciences, University of Windsor, Windsor, Ontario, Canada*

Rhinotopy describes the observation that olfactory sensory neurons in a particular zone of the olfactory epithelium innervate a distinct region of the olfactory bulb (Schoenfeld et al., 1994 Brain Res. Bull. 34: 183-210). This phenomenon is thought to function in the organization of olfactory information. Here we seek to investigate if rhinotopy occurs in the sea lamprey, Petromyzon marinus, an ancestral jawless fish with migratory and reproductive behavior mediated through pheromones (Li et al., 1995 J. Gen. Physiol. 105: 567; Li et al., Science 2002 296: 138-141). Our previous studies have shown that in the olfactory bulb, medial glomeruli differ from the other glomeruli, in that they contain 5HT innervation, but do not express Golf. Therefore, we were interested in determining if olfactory sensory neurons that project to these medial glomeruli originate from spatially distinct regions of the olfactory epithelium. Our method of investigation was through micro-injecting 3000 MW fluorescent dextran amines into specific glomerular territories of the olfactory bulb. The dye fills the olfactory sensory neurons through retrograde movement via the olfactory nerve into the olfactory epithelium. Injections into the medial glomeruli have displayed olfactory sensory neurons with dendrites and soma concentrating in lateral and medial regions of the olfactory epithelium. Further investigation using immunocytochemical and physiological techniques is required in order to elucidate a possible role for this pathway in odorant decoding.

100 Poster: Olfactory CNS Processing

ULTRAVIOLET (UV) LIGHT MODIFIES SALAMANDER OLFACTORY EPITHELIAL (OE) AND OLFACTORY BULB (OB) RESPONSES TO ODORANT STIMULI

Cheung M.[1], Kauer J.S.[1] [1]*Neuroscience, Tufts University, Boston, MA*

We have previously reported that OE-exposure to ultraviolet (UV) light at a normalized intensity similar to ~2.6% of full-spectrum sunlight causes suppression of frog olfactory sensory neuron (OSN) responses as measured by the electro-olfactogram (EOG) (Cheung and Kauer, AChemS XXIV). Here we demonstrate that OSN response blockade by UV-light in both frog and salamander also alters odorant-elicited responses in the glomerular, mitral/tufted cell, and granule cell layers in the OB observed using real-time voltage sensitive dye (VSD) recording with di-4-ANEPPS (Molecular Probes, Oregon, USA). Suppressing the entire OE in tiger salamander with UV-light irradiation eliminates OB responses to amyl acetate, gerianol, acetophenone, and ethyl fenchol. Electrically stimulating the olfactory nerve (ON) elicited bulbar activity both before and after UV-exposure of the OE and indicates that only the odorant initiated OSN events are affected by UV. These findings demonstrate a means by which OSN responses can be altered in spatially defined ways. Marchand et al. (AChemS XXIII) have demonstrated that tiger salamander OSNs express olfactory receptor transcripts in restricted and defined regions of the OE. Since OSNs that express a single receptor type converge onto one or a few glomeruli, spatially defining regions of OE for UV-exposure should also restrict the set of responding glomeruli. Our hypothesis is that we will be able to suppress selected populations of OSNs in different OE regions so we can examine how each contributes to the bulbar response patterns generated by different odorant stimuli. Supported by NIDCD.

101 Poster: Olfactory CNS Processing

I_H MODULATION OF GRANULE CELL FUNCTION IN THE MOUSE MAIN OLFACTORY BULB

Aungst J.L.[1], Heyward P.M.[2], Hayar A.M.[2], Shipley M.T.[2] [1]*Anatomy and Neurobiology, University of Maryland at Baltimore, Baltimore, MD;* [2]*Anatomy and Neurobiology, Program in Neuroscience, University of Maryland at Baltimore, Baltimore, MD*

Interactions between main olfactory bulb (MOB) mitral cells (MC) and granule cells (GC) through reciprocal dendrodendritic synapses are important for olfactory function. Modulation of GC membrane properties greatly influences their responses to synaptic input and interactions with mitral cells. Granule cells express the anomalous rectifier current, I_H, an inward current activated by hyperpolarization. Preliminary evidence suggests that activation of α2 receptors attenuates I_H in GCs. The goal of this study was to characterize the IH current in GCs and determine its role in modulating the MC-GC circuit. Somatic whole cell current clamp recordings of GCs in mouse MOB horizontal slices showed heterogeneity in the strength of I_H following hyperpolarizing current injections. Somatic hyperpolarization activated a robust I_H current, blocked by extracellular cesium, in approximately 50% of GCs. However, in all GCs cesium application increased membrane resistance. These data suggest that IH is present in all GCs, but may be located remote to the somatic recording site in GC dendrites and unmasked by cesium treatment. We are investigating the relationship between GC dendritic structure and the magnitude of I_H, in recorded GCs. Dendritic localization of I_H currents would make this current highly effective at altering MC-GC synaptic communication.

102 Poster: Olfactory CNS Processing

NEURAL BASIS FOR INTERGLOMERULAR CIRCUITS IN THE MAIN OLFACTORY BULB.

Puche A.C.[1], Aungst J.L.[1], Heyward P.M.[1], Erdelyi F.[2], Szabo G.[2], Karnup S.V.[1], Shipley M.T.[1] *[1]Dept. Anatomy and Neurobiology, Program in Neuroscience, University of Maryland at Baltimore, Baltimore, MD; [2]Dept. of Gene Technology and Developmental Neurobiology, Institute of Experimental Medicine, Budapest, Hungary*

Lateral interactions at the level of the glomerular layer are likely to be critical for processing information in the main olfactory bulb (MOB). A primary candidate for mediating lateral interactions between glomeruli is the short axon cell, which is thought to express GABA and project only 2-3 glomeruli distant. To investigate the distribution of interglomerular projections, small DiI or latex microsphere injections were made into the glomerular layer of both mouse and rat. These injections labeled numerous juxtaglomerular (JG) cells with processes coursing laterally through multiple glomeruli millimeters from the injection site. Reconstructed individual biocytin filled short axon cells had multiple processes projecting over similar distances. The expression of GABA in these cells was investigated using dye injections into the glomerular layer of transgenic mouse expressing the green fluorescent protein under the control of the GAD65 promoter. Surprisingly, only ~8% of dye labeled JG cells expressed GAD65. Using both voltage sensitive dye imaging in a novel surface slice preparation, and whole cell recordings of JG cells, we show that the majority of interglomerular connections are excitatory and dependent on glutamate release. Taken together, these results demonstrate that interglomerular connections project over longer distances than previously believed and are principally excitatory. Supported by NIH. DC02173, DC00347 & NS36940

103 Poster: Olfactory CNS Processing

SELECTIVE ACTIVATION OF CA INFLUX THROUGH NMDA RECEPTORS IN THE MITRAL CELL GLOMERULAR TUFT

Xiong W.[1], Chen W.R.[1] *[1]Neurobiology, Yale University, New Haven, Connecticut*

Mitral cell distal glomerular tuft presents an independent functional unit for the transmission and processing of olfactory signals. Besides receiving synaptic input from olfactory nerve (ON) terminals, it also produces dendrodendritic output to periglomerular interneurons. To analyze this close input-output coupling, we have applied patch-clamp recording and Ca imaging to the rat olfactory bulb slices. In response to increasing ON stimulation, the mitral cell glomerular tuft showed an all-or-none-like Ca response which corresponded to the threshold of action potential initiation at the soma. This suggests that Ca increase in the glomerular tuft is primarily mediated by Ca channels activated by the back-propagating action potential rather than by postsynaptic NMDA receptors. However, when the somatic spike was suppressed either by DC hyperpolarization injected at the soma or by IPSP induced in secondary dendrites, the curve of Ca response over stimulus intensity was no longer step-wise but instead displayed an initial ramp followed by a sudden jump. The later jump was due to dendritic action-potential initiation with strong ON stimulation, while NMDA receptor antagonist (50 μM APV) completely blocked the initial ramp and converted the response curve back into a step-wise change. In the ramp-response range, Ca increase was confined only to the glomerular tuft and its rising time course was much slower than that evoked by action potentials. These results indicate that, with normal Mg concentration, Ca influx via NMDA receptors can be selectively activated without contamination of voltage-gated Ca channels, and may play a unique role in glomerular transmission and plasticity. Supported by NIH grant (DC-03918).

104 Poster: Olfactory CNS Processing

ARE GLUTAMATE SPILLOVER AND NMDA AUTOCEPTORS THE KEY MECHANISM FOR GLOMERULUS-ASSOCIATED NEURONAL SYNCHRONY?

Chen W.R.[1], Xiong W.[1], Zhou Z.[1] *[1]Neurobiology, Yale University, New Haven, CT*

Excitation synchrony among mitral cells that project to a common glomerulus represents a prominent feature of the olfactory bulb circuits, and is believed to be critically involved in olfactory coding and processing (Carlson et al., 2000; Schoppa & Westbrook, 2001; Puopolo & Belluzzi, 2001). Neuronal oscillation and synchronization in the olfactory bulb is widely assumed to be mediated by glutamate spillover and NMDA autorceptors (Isaacson, 1999; Schoppa & Westbrook, 2001). By recording simultaneously from pairs of juxta-glomerular (JG) cells that innervate a common glomerulus, we observed large periodic depolarizations occurring spontaneously in normal extracellular medium containing 1.3 mM magnesium ions. These slow oscillations recorded from different JG cells were often in phase with each other, and appeared to reflect synchronized excitation of glomerulus-associated cell population. Our detailed analysis of these periodic depolarizations reveals that, with the presence of normal concentration of magnesium ions, glutamate spillover and NMDA autoceptors are not essentially involved in the generation of glomerulus-specific neuronal synchrony. Supported by NIH grant (DC-03918).

105 Poster: Olfactory CNS Processing

PRESYNAPTIC INHIBITION MODULATES THE AMPLITUDE AND TIME COURSE OF THE ODORANT ELICITED INPUT TO THE MOUSE OLFACTORY BULB

Vucinic D.[1], Cohen L.B.[1], Kosmidis E.[1] *[1]Cellular & Molecular Physiology, Yale University, New Haven, CT*

Previous experiments on in vitro preparations from the turtle and mouse (e.g. Wachowiak and Cohen, 1999; Ennis et al, 2001; Wachowiak et al, 2002) showed that presynaptic inhibition acts on the olfactory receptor neuron nerve terminals via $GABA_B$ and dopamine D_2 receptors. In in vivo C57Bl/6 mice we have used the fluorescence signal from Calcium Green-1 dextran in these nerve terminals to monitor the input to the olfactory bulb in response to odorant presentations to the nose. We applied agonists and antagonists of the $GABA_B$ and dopamine D_2 receptors to the olfactory bulb and tested whether these agents affected the size, the time course, or the map of the input to the bulb. In most preparations the $GABA_B$ agonist, baclofen, and the dopamine D_2 agonist, quinpirole, reduced the amplitude of the Calcium increase in response to odorants by 20-60%. In contrast, the $GABA_B$ antagonist, CGP46381, increased the size of the Calcium signal by 10-50%. Both the $GABA_B$ agonist, baclofen, and the $GABA_B$ antagonist, CGP46381, also slowed the time course of the Calcium signal. In contrast, neither the dopamine D_2 agonist, quinpirole, nor the antagonist, sulpiride, seemed to have an effect on the time course of the Calcium increase. Thus, the two presynaptic mechanisms may play different roles in olfactory processing. A preliminary examination to determine whether the maps of input to the bulb were altered by presynaptic inhibition suggests that such alterations are either small or absent. Supported by NIH grants number NS07455 and DC05259.

106 Poster: Olfactory CNS Processing

IS ENERGY NOT A CONCERN FOR BULBAR ACTIVITY?

Hyder F.[1] [1]*Diagnostic Radiology, Yale University, New Haven, CT*

Since glucose is the main and almost exclusive carbon source for the central nervous system, glucose oxidation is the main energy producing pathway. The human brain comprising of only about 2% of the body mass accounts for approximately 20% of the total oxygen consumption in the body. The extremely high energy demand of the human brain and its relation to cerebral function forms a fascinating research narrative of recent past. The brain's large energy budget as well as its stimulus-defined usage of localized energy has lead to reinterpretation of neuroimaging data. This level of biophysical understanding of bulbar activity is greatly lacking. Quantitative functional magnetic resonance imaging (fMRI) experiments conducted in both the rat brain and olfactory bulb reveal that stimulus-evoked changes (by forepaw and odorant stimulations, respectively) in localized energy use are about 3-4 times higher in the olfactory bulb. Since the olfactory bulb in the rodent is typically about 1/10 the size of the brain by weight, the comparison of quantitative fMRI results from brain and olfactory bulb suggest that the latter is a significantly more energy demanding organ. Although the olfactory bulb comprises of only about 0.2% of the body mass in the rodent, it may account for about 10% of the total oxygen consumption in the rodent. Since the cumulative use of oxidative energy in the rat brain and olfactory bulb may account for as much as 30% of the total oxygen consumption in the body, we propose that issues of energy budget should be considered for interpreting neuroimaging data of the olfactory bulb. Supported by NIH (NS-037203, DC-003710, MH-067528) and NSF (DBI-0095173) grants.

107 Poster: Olfactory CNS Processing

CHARACTERIZATION OF THE SYNAPTIC PROPERTIES OF OLFACTORY BULB PROJECTIONS

McNamara A.[1], Cleland T.A.[2], Linster C.[1] [1]*Cornell University*, Ithaca, New York;* [2]*Cornell University, Ithaca, New York*

Olfactory bulb mitral cells project to several telencephalic structures, including the anterior olfactory nucleus, several rostromedial cortices including the ventral tenia tecta, anterior hippocampal continuation, and indusium griseum, the subpallial olfactory tubercle, the piriform and entorhinal cortices, and the anterior cortical nucleus of the amygdala. To date, few studies have investigated the odor processing characteristics of these anatomically diverse structures, other than the piriform cortex. We have performed in vivo electrophysiological experiments in urethane-anesthetized Sprague-Dawley rats to characterize the synaptic properties of the bulbar projections to these structures. Using paired pulse stimulation of the lateral olfactory tract (25 to 1000 ms inter-pulse intervals), we observed synaptic facilitation to the second pulse in all target structures, but found that the time course and degree of this facilitation varied considerably among structures. Similarly, synaptic facilitation was also observed during pulse train stimulation (twenty-pulse trains with inter-pulse intervals varying between 25 and 500 ms), but the rise time, steady-state peak, and decay time of facilitation differed among the target structures. Supported by Alfred P. Sloan Foundation (CL)

108 Poster : Olfactory CNS Processing

RATE-ENCODING VERSUS TEMPORAL-ENCODING OF NEURAL ACTIVITY IN HUMAN PRIMARY OLFACTORY CORTEX

Johnson B.N.[1], Mainland J.D.[2], Bensafi M.[2], Khan R.M.[2], Bremner E.[2], Sobel N.[2] [1]*Bioengineering, University of California, Berkeley;* [2]*Neuroscience, University of California, Berkeley*

Functional magnetic resonance imaging (fMRI) of primary olfactory cortex (POC) has yielded inconsistent results. Odorant-induced POC activity is present at times and absent at others even within the same lab using the same task. Most statistical models used to analyze fMRI data rely on two assumptions: 1. a monotonic transform from stimulus magnitude to neural activity quantity, and 2. a linear transform from neural activity quantity to MR signal magnitude. Whereas the latter has been demonstrated for MR (Boynton et al 1996), the former has not been demonstrated for POC. An equally viable alternative to a rate-encoding model is a temporal encoding model. Models of temporal encoding imply no monotonic transform from stimulus magnitude to neural activity quantity. Thus, fMRI is a potentially invalid measure of POC activity under temporal encoding models. To address this issue we set out to quantify the stimulus magnitude dependence in POC in thirty subjects. An olfactometer generated low, medium and high concentrations of the odorants valeric acid, phenethyl alcohol, and propionic acid in an event-related design (4T, T2* GEMS, TE=28ms, TR=1000ms, 192mm FOV, 8 slices, 0.5mm skip, 3x3x3.5mm voxel, ISI=30sec, stimulus repetition=27). For the initial 6 subjects of propionic acid there was significant activation overall in lateral orbital fronal cortex ($p<.05$) and an overall trend towards increased fMRI signal with increased stimulus magnitude in POC. In several of these initial subjects the signal in POC appeared to be confounded by individual sniff dynamics. Final data analysis will include regressors based on individual sniffs to reduce this variability. Funded by SOSI & NIH-NIDCD R03-DC05141-01

109 Poster : Olfactory CNS Processing

COORDINATED SYNAPTIC MECHANISMS UNDERLIE CORTICAL OLFACTORY ADAPTATION

Best A.R.[1], Wilson D.A.[2] [1]*Zoology, University of Oklahoma, Norman, OK;* [2]*Department of Zoology, University of Oklahoma, Norman, OK*

Anterior piriform cortex (aPCX) 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 (1-2 minutes) associated with this odor habituation, in vivo. 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 to record LOT-evoked responses from layer Ia (fEPSP's) or layer II/III neurons (EPSP's). Extracellular and intracellular 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 to mimic odor input). Results from this paradigm were compared with in vivo intracellular recordings from layer II/III neurons within aPCX of urethane-anesthetized rats stimulated with odorants. The time-course and extent of LOT synaptic depression induced by both in vitro electrical and in vivo odorant stimulation methods were similar. There was no evidence of heterosynaptic depression between independent inputs in either model. In vitro evidence suggests that at least two mechanisms may underlie this activity-dependent synaptic depression. A rapidly recovering pre-synaptic vesicle depletion seems to dominate the initial 10-20s of the effect. A longer lasting (~100s) depression also exists that can be decreased by either adenosine or L-AP4. Supported by: DC03906 to DAW

110 Poster : Olfactory CNS Processing

MODULATION OF OLFACTORY CORTEX FUNCTION BY CORTICAL ASSOCIATION FIBERS: INTRACELLULAR ANALYSIS IN VIVO

Gilmore H.L.[1], Stripling J.S.[1] [1]*Psychology, University of Arkansas, Fayetteville, AR*

The role of cortical association fibers (AF) in piriform cortex (PC) function was investigated using intracellular recordings from identified PC neurons in freely-breathing male Long-Evans rats under urethane anesthesia (1.4 g/kg i.p.). PC neurons exhibited a marked fluctuation in membrane potential that was synchronized with respiration. High-frequency stimulation (HFS) of AF (10-pulse 100-Hz trains) produced a substantial hyperpolarizing shift in membrane potential (mean = -6 mV) that decayed gradually over 10-30 sec following HFS. Intracellular potentials evoked by single-pulse stimulation of AF or the lateral olfactory tract (LOT) were differentially affected following HFS of AF. The AF-evoked potential exhibited a pronounced increase in the amplitude of an early depolarizing component. Reversal potential analysis indicated that the potentiated component was strongly voltage-dependent and appeared to be a depolarizing Cl- IPSP that was augmented due to the increased driving force on Cl- produced by hyperpolarization. A similar potentiation occurred during the hyperpolarizing phase of naturally-occurring voltage fluctuations driven by respiration. In contrast, the LOT-evoked potential exhibited little voltage-dependence and was minimally affected following HFS of AF. These results indicate that patterned activation of AF in vivo produces inhibitory effects that may increase the signal-to-noise ratio in olfactory cortex by suppressing ongoing background activity. Supported by: NIH grant DC02271 and the Marie Wilson Howells Fund.

111 Poster : Olfactory CNS Processing

IMPAIRED LTP IN OLFACTORY CORTEX OF MICE LACKING THE FRAGILE X MENTAL RETARDATION PROTEIN

Larson J.[1] [1]*Psychiatry, University of Illinois at Chicago, Chicago, Illinois*

Fragile X syndrome, the most common inherited form of mental retardation, is caused by mutation of the gene encoding the fragile X mental retardation protein (FMRP). Knockout (KO) mice lacking FMRP have been developed as models of the disease. The present study tested for alterations in long-term potentiation (LTP) in primary olfactory cortex of FMRP-deficient mice Slices of anterior piriform cortex were prepared from KO and wild-type (WT) mice (C57BL/6J strain) and maintained in vitro using conventional methods. Synaptic responses evoked by associational fiber stimulation were recorded extracellularly in layer Ib. Input-output curves were generated by varying stimulus intensity from 2.5 to 160 uA, paired-pulse stimulation used inter-pulse intervals of 50 to 1600 msec, and LTP was induced by theta burst stimulation (TBS). KO mice had apparently normal excitatory transmission (input-output curves) and short-term synaptic plasticity (paired-pulse depression) in piriform cortex slices. However, LTP induced by TBS was substantially smaller in FMRP-deficient mice than in the controls. The percent potentiation 60 min. after TBS was 5.3 $\pm$2.9 in KO (n=7) slices and 22.2 $\pm$4.7 in WT (n=8) slices ($t=2.97$, $p<.02$). Slices from KO mice showed normal responses to TBS but the resultant potentiation decayed significantly over time, unlike control slices, which showed stable LTP. These results indicate that FMRP contributes to mechanisms of long-term synaptic potentiation in primary olfactory cortex and suggest that a defect in cortical synaptic plasticity contributes to learning disabilities in fragile X syndrome. (Supported by FRAXA Research Foundation.)

112 Poster : Olfactory CNS Processing

CEREBELLAR ROLE IN OLFACTION: CONCENTRATION DEPENDANT SNIFF MODULATION

Mainland J.D.[1], Telles C.[1], Amin N.[1], Johnson B.N.[2], Bremner E.A.[1], Bensafi M.[1], Khan R.M.[1], Ivry R.B.[1], Sobel N.[1] [1]*Neuroscience, University of California, Berkeley, Berkeley, CA;* [2]*Bioengineering, University of California, Berkeley, Berkeley, CA*

Nearly every olfaction imaging study to date has reported odorant-induced activation of the cerebellum, however its role in olfaction is unknown. In vision, audition, and somatosensation the cerebellum has been hypothesized to subserve precise online sensory-motor coordination. In olfaction, we hypothesize that this would translate as modulation of the sniff in response to odor concentration. To test this theory we intend to use three approaches: functional magnetic resonance imaging (fMRI), transcranial magnetic stimulation (TMS), and lesion patients. We examined 8 patients with cerebellar lesions. In agreement with the predicted role of the cerebellum in olfaction, correlations between sniff volume and odorant concentration were weaker than controls ($p<0.05$). Six of the patients had unilateral lesions, and showed stronger correlations when odor was delivered to the ipsilesional nostril than the contralesional nostril ($p<0.04$). Anatomical connections between olfactory inputs and the cerebellum are not well understood, so functional connectivity was assessed with fMRI during unilateral odorant delivery. Contrary to what we would expect from the lesion results, there was a trend showing the cerebellar cortex being primarily driven by ipsilateral odor delivery ($p<0.12$). More subjects will be run to test this trend. To resolve this discrepancy, transcranial magnetic stimulation (TMS) will be used over the cerebellum of 12 healthy subjects while performing an olfactory task to probe the time course and laterality of cerebellar involvement in the regulation of sniff volume. Funded by NIH NIDCD

113 Poster : Retronasal Olfaction

ANALYSIS OF FOOD FLAVORS BY GAS CHROMATOGRAPHY-RETRONASAL OLFACTOMETRY

Honma Y.[1], Komatsu H.[2], Higashi N.[3], Shikata H.[3] [1]*Tobacco Science Research Center, Japan Tobacco Inc., Yokohama, E, PY, PR, Japan;* [2]*Leaf Tobacco Research Laboratory, Japan Tobacco Inc., Oyama, C, Japan;* [3]*Tobacco Science Research Center, Japan Tobacco Inc., Yokohama, C, Japan*

Some studies have reported the differences of odor quality/intensity for the same odorant between orthonasal and retronasal olfaction. These differences could have been caused by moisture in the oral cavity, partition of odor compounds to saliva and oral epithelium, enhanced volatility by chewing and body temperature, and profile of nasal air flow. In the research and design of food flavor, we have to be aware of these differences to understand the effect of each component to overall flavor perception. In flavor research, Gas chromatography-Olfactometry (GC-O) techniques are frequently employed, as a valuable technique for detecting odor compounds in a sample matrix. However, all studies have been performed by only orthonasal olfaction, which detects odorous components by "sniffing". For the reason mentioned above, we tried to apply retronasal olfaction to GC-O technique and compared the differences in odor perception with orthonasal olfaction. Therefore we "puffed" the odorous components eluted from the GC into the mouth and made an "aromagram" which indicates the odor quality/intensity of the compounds in a sample. As results, aromagram by retronasal olfaction generally showed the less sensitivity and a poor discrimination ability for most of the odorous components compared to that of orthonasal olfaction. However we found exceptions for some components, for example enhancement of sensitivity and remarkable change of odor quality.

114 Poster : Retronasal Olfaction

THE EFFECT OF LEARNING MODALITY ON THE RETRONASAL IDENTIFICATION OF ODORS IN ODOR-TASTE MIXTURES

Wilkes F.[1], Laing D.G.[1], Jinks A.[1], Hutchinson I.[1] *[1]Centre For Advanced Food Research, University of Western Sydney, Richmond, Australia*

Odors released into the nose can smell differently when released into the mouth. The existence of a gating mechanism in the brain that controls the storage and later access to odor information could answer this question (Rozin 1982). Thus, odor information learned via the nose may rely on the correct triggering of the gating mechanism to allow future access to this information. Here a selective attention paradigm investigated whether the mechanism was based on the modality of odor learning and odor familiarity. The 3 learning modalities were mouth-familiar (using the odors, orange, cinnamon, fish), nose-familiar (grassy, floral, nail polish remover) and unfamiliar (octanol, nonanone, gamma-valerolactone). It was expected that the gating mechanism would result in mouth-familiar odors being better identified than nose-familiar and unfamiliar odors. If the mechanism relies on modality, nose-familiar and unfamiliar odors would be identified at levels at or near chance, as the correct triggering of the gating mechanism would not occur. The results indicated that when presented in the mouth in a binary mixture with a tastant, mouth-learned odors were identified better than nose-learned and unfamiliar odors. Hence, if a gating mechanism exists, it does not rely on the presentation of an odor through the modality that it was learned for it to be correctly identified, as all odors were identified above the chance level (50%). However, presenting a mouth-familiar odor via the mouth increases the accuracy of identification suggesting that context and learning experience are important factors.

115 Poster : Retronasal Olfaction

MODELING OLFACTORY PERCEPTION WITH A PERCEPTION-BASED TEMPLATE IMPROVES FMRI DATA PROCESSING

Cerf-Ducastel B.[1], Murphy C.[1] *[1]Psychology, San Diego State University, San Diego, California*

Eight young subjects participated in a functional Magnetic Resonance Imaging (fMRI) study of retronasal olfactory perception induced by odorants dissolved in water and presented to the mouth. Stimuli were presented as boluses of 50 microl every 3 s in blocks of 18 s alternating with water for 75 s. FMRI data were collected with a 1.5T scanner (Siemens, EPI sagittal functional images, 4x4x4 mm3, Mprage structural image, 1x1x1 mm3). A region of interest (ROI) analysis was conducted with AFNI software on 12 ROIs in each hemisphere, selected from the Talairach database. The average signal from each ROI was correlated with two templates: (i) a template based on the stimulation paradigm (block design convolved with a hemodynamic function) and (ii) a template based on the average perception profile collected on young subjects. Repeated measures ANOVA on Fisher transformed correlation coefficients showed a main effect of template for regions critical to olfactory processing (i.e. amygdala, entorhinal cortex, hippocampus, insula, posterolateral orbitofrontal cortex, piriform cortex, parahippocampal gyrus). On the other hand, no significant effect of template was observed for other areas (i.e. anterior and posterior cingulated gyri, anteromedial orbitofrontal cortex, superior and inferior occipital gyri) covering the same number of voxels, suggesting an olfactory specific effect. These results suggest that brain responses to retronasal olfactory stimulation might be better modeled by a perception based template than by a stimulation based template. Supported by NIH grant AG04085 from the National Institute on Aging to CM.

116 Poster : Retronasal Olfaction

TIME-QUALITY TRACKING OF RETRONASAL AND ORTHONASAL ODORANT PAIRS.

Chaudhry K.[1], Halpern B.P.[2] *[1]Neurobiology & Behavior, Cornell University, Ithaca, NY; [2]Psychology and Neurobiology & Behavior, Cornell University, Ithaca, New York*

Using odorant presentation containers (OPC; Pierce and Halpern, Chem. Senses, 21, 529-543, 1996), subjects 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 to 100% correct, retro, and ortho, veridical names of the matched concentrations and of the orange standard. In a later session, subjects used a computer-mouse to indicate what they smelled over time by selecting on timed computer displays the names of the six odorants, or "no odor", in response to OPC-presented randomized vapor-phase ortho and retro pairs of all odorants, and to all odorants paired with empty OPC. Subject either initially exhaled (retro 1st) or inhaled (ortho 1st). Subjects differed in the matching retronasal and orthonasal concentrations they chose. Subjects could identify odors while smelling them, but with large individual differences. Both retro and ortho odorants were identified by some individuals, sometimes through several breathing cycles; reports of "no odor" following the first identification were rare. Other individuals generally identified only the odorant smelled 1st. Support from USDA grant 2001-355503-10102.

117 Poster : Retronasal Olfaction

RETRONASAL OLFACTORY INTENSITY: ASSOCIATIONS WITH TASTE

Duffy V.B.[1], Chapo A.K.[2], Hutchins H.L.[1], Snyder D.[2], Bartoshuk L.M.[2] *[1]Dietetics, University of Connecticut, Storrs, CT; [2]Surgery, Yale University, New Haven, CT*

Bull (1965) reported alterations in oral sensations with surgical damage to the chorda tympani nerve (CTN) including those suggesting loss of retronasal olfaction. Changes to localization of retronasal olfaction occur by manipulating taste and touch through CTN anesthesia alone (Fast et al, 2000) or with the lingual nerve (Snyder et al, 2001). Here two studies show associations between retronasal olfaction and taste including probes of taste genetics: propylthiouracil (PROP) bitterness and fungiform papillae number (FP). Jellybeans were sampled with the nose pinched (primarily sweet: JB taste) and unpinched (retronasal olfaction and sweet: JB flavor). The general labeled magnitude scale was used to measure perceived intensity. In a laboratory study, JB flavor was predicted in 35 aged women from JB taste, PROP, FP, QHCl on the tongue tip (CTN pathology), and orthonasal olfaction (threshold, odor identification; Cain et al, 1988). All but FP were significant predictors in multiple regression. Greater orthonasal function was associated with greater JB flavor but not with other taste measures. Those with greater JB taste or PROP bitterness had greater JB flavor; those with lower tongue tip QHCl had lower JB flavor. In a screening study, JB flavor was predicted from JB taste, PROP and FP in 245 adults (22 to 55 years). In multiple regression analysis, greater JB taste and higher FP were significant predictors of greater JB flavor. These studies suggest contributions of taste and possibly touch sensations to retronasal intensity, independent of olfactory function. Retronasal olfaction may be heightened in genetic supertasters and diminished in those with pathologies that damage the CTN. (NIA RO3AG18619, NRICGP/USDA 2002-00788 and DC00168 funded)

118 Poster : Retronasal Olfaction

ORTHONASAL VS. RETRONASAL ODORANT ADMINISTRATION DIFFERENTIALLY AFFECTS ATHLETIC PERFORMANCE, MOOD, AND WORKLOAD

Zoladz P.[1], Raudenbush B.[1], Fronchoski R.[1], Price N.[1] *[1]Psychology, Wheeling Jesuit University, Wheeling, West Virginia*

Past research has shown that orthonasal administration of peppermint odor facilitates athletic performance, mood, and workload. Raudenbush, Meyer, and Eppich (2002) had 40 athletes perform a modified 15-minute treadmill exercise under four odorant conditions (peppermint, jasmine, dimethyl sulfide, or a non-odored control condition). During testing, pre- and post-physiological measures were recorded, as well as subjective measures of mood and workload. Raudenbush and colleagues found that peppermint odor significantly reduces athletes perceived workload and levels of fatigue. Additionally, subjective evaluations of athletic performance were significantly greater in the presence of the peppermint odor, and athletes rated their level of vigor higher in the peppermint condition. In contrast, the present study revealed that retronasal administration of peppermint odor (via chewing gum) does not significantly affect athletic performance, mood, or workload. In this study, 20 collegiate athletes performed the same modified 15-minute treadmill exercise as used in the Raudenbush et al. (2002) study under each of five chewing gum conditions (peppermint gum, cinnamon gum, fruity gum, flavorless gum, or a no gum control condition). Again, during testing, pre- and post-physiological measures were recorded, as well as subjective measures of mood and perceived workload. Results showed that no chewing gum condition, including peppermint, significantly affected athletic performance, mood, or workload. The implications of such findings are important, as they suggest a differential effect of peppermint odor on athletic performance through orthonasal administration versus retronasal administration.

119 Poster : Retronasal Olfaction

ORTHO- AND RETRONASAL PERCEPTION OF ODORS: A STUDY BASED ON EVENT-RELATED POTENTIALS

Hummel T.[1], Heilmann S.[1] *[1]Smell and Taste Clinic, Otorhinolaryngology, University of Dresden Medical School, Dresden, Germany*

The current study aimed to investigate differences between ortho- and retronasal perception using olfactory evoked potentials. A total of 24 subjects (12 women, 12 men, age 19 – 45 years) participated. In addition to examination of orthonasal olfactory function using the "Sniffin´ Sticks" test battery (phenyl ethyl alcohol odor threshold, odor discrimination, and odor identification) the subject´s retronasal olfactory function was assessed psychophysically using an identification test consisting of 20 aromas applied orally. Olfactory event-related potentials (OERP) were recorded in response to ortho- and retronasal stimulation. For retronasal stimulation odorants were applied via tubing placed below the lower turbinate under endoscopic control. Odorants were thus released in the epipharynx. For olfactory stimulation phenyl ethyl alcohol and hydrogen sulphide where used in two different concentrations each. Preliminary analysis of the data indicated that OERP to ortho- and retronasal stimulation exhibited a good correlation. OERP to both ortho- and retronasal stimulation discriminated between stimulus intensities. Effects in relation to the site of stimulation were found for N1 latency, which was shorter after orthonasal stimulation. These investigations indicate, that OERP are suitable for the investigation of cortical information processing in relation to ortho- and retronasal stimulation. The responses to ortho- and retronasal stimulation appeared to be similar in many respects. However, the presently observed differences for N1 latencies indicate different mechanisms of information processing depending on the site of olfactory stimulation.

120 Poster : Retronasal Olfaction

COMPARISON OF ORTHONASAL AND RETRONASAL PERCEPTION OF NON-FOOD ODORS: A FUNCTIONAL MR IMAGING STUDY

Gerber J.C.[1], Small D.[2], Heilmann S.[3], Hummel T.[3] *[1]Neuroradiology, University of Dresden, Germany; [2]Northwestern University Medical School, Chicago, IL; [3]ORL, University of Dresden, Dresden, Germany*

Aim was to investigate the cortical representation of olfactory perception in response to ortho- and retronasal stimulation using functional MR imaging (fMRI). In this preliminary study 4 healthy subjects underwent fMRI. Data were analysed using SPM99. Olfactory stimulation was performed with phenyl ethyl alcohol and H2S (block design, 30s on, 30s off; 1s stimulus duration, 4s interval); stimuli were presented to the anterior nasal cavity or to the epipharynx using special canulas. On first approximation, ortho- and retronasal stimulation produced a similar pattern of activation when compared to their respective baseline conditions. Irrespective of presentation mode, a group conjunction analysis showed activation in the insula (R>L), anterior cingulate, superior temporal gyri, and right lateral orbital gyrus. However, when evaluating differences in relation to stimulus presentation, the left insula and anterior cingulate had higher activation following orthonasal stimulation. Using the same contrast in the analysis of individual subjects the right caudate nucleus (2/4) and medial parts of the right thalamus (2/4) showed activation. No significant differences were observed in the reverse contrast (retro–ortho). In conclusion, cortical activation patterns following ortho- and retronasal stimulation show great overlap. However, areas specifically tied to the processing of ortho- or retronasal stimuli may be suspected in the insula, cingulate gyrus, caudate nucleus, and thalamus. Support: DFG HU441-2

121 Poster : Retronasal Olfaction

RETRONASAL PRESENTATION OF A FOOD ODOR PREFERENTIALLY ACTIVATES CORTICAL CHEMOSENSORY AREAS COMPARED TO ORTHONASAL PRESENTATION OF THE SAME ODOR AND RETRONASAL PRESENTATION OF A NONFOOD ODOR.

Léger G.C.[1], Hummel T.[2], Conley D.B.[3], Mak E.Y.[4], Simmons K.[4], Small D.[5] *[1]Neurology, Northwestern University, Chicago, IL; [2]University of Dresden Medical School, Germany; [3]Otorhinolaryngology, Northwestern University; [4]Northwestern University, Chicago, IL; [5]Northwestern University Medical School*

In 1982 Rozin proposed that olfaction could be divided into two distinct senses; orthonasal olfaction for identifying objects at a distance and retronasal olfaction for identifying food in the mouth. In support of this notion we show differential processing of food (maple) and non-food (lavender) odors dependent upon mode of stimulation (ortho vs retro). Subjects underwent fMRI scanning with a Siemens 1.5T magnet while they were randomly exposed to 6 conditions (maple retro, maple ortho, lavender retro, lavender ortho, clean air retro, clean air ortho) in a block-design paradigm. All stimuli were delivered as vapor for 30 seconds to either the anterior nasal cavity or to the epipharynx using special canulas. Importantly, food odors will have been frequently experienced via both retronasal and orthonasal routes whereas non-food odors will have been experienced predominantly via the orthonasal route. Group analysis was performed with SPM99. Comparison of each odor condition minus the appropriate clean air baseline produced activation in the orbitofrontal cortex (OFC). This region was also activated by retronasal compared to orthonasal stimulation in both odor categories, but significantly greater activation was observed during retronasal maple stimulation compared to retronasal lavender stimulation. Supported by a research grant awarded to DM Small by the Unilever Corporate Research Program, and a grant awarded to T Hummel (DFG HU441 2-1).

122 Poster : Retronasal Olfaction

RETRONASAL OLFACTION IN NASAL POLYPOSIS

Landis B.N.[1], Giger R.[1], Hugentobler M.[1], Hummel T.[2], Lacroix J.[1]
[1]ORL, Hopitaux Universitaires de Geneve, Geneva, Switzerland; [2]Smell and Taste Clinic, Otorhinolaryngology, University of Dresden Medical School, Dresden, Germany

Objective: To test retro- versus orthonasal olfactory identification (OlfId) function in nasal polyposis (NP) patients and healthy controls.

Study Design: Open prospective study comparing NP patients versus a healthy control group. Methods: 56 healthy volunteers and 42 NP patients were tested with a orthonasal and retronasal smell identification test. Subjects had nasal endoscopy and NP was staged according the Malm classification. Patients rated their olfactory function on visual analogic scales. Orthonasal OlfId was done by means of the "Sniffin' Sticks". Retronasal OlfId was evaluated with commercially available food powders applied on the oral cavity. Both tests consisted of a ten items forced choice smell identification. Results: Total OlfId was better in healthy controls compared to NP patients. Within the groups, healthy controls showed no difference in orthonasal versus retronasal OlfId, whereas NP patients showed significant better retronasal compared to orthonasal olfactory function. This difference in orthonasal and retronasal OlfId was only present in NP stage 1 and 2. Ratings of olfactory function correlated highly with retronasal and orthonasal OlfId in NP patients, but not in healthy controls. Conclusion: Better retro- than orthonasal OlfId seems to be associated with the presence of anterior mechanical obstruction towards the olfactory cleft typically observed in NP. Clinically these patients present themselves with smell loss without taste complaints. Our work suggests that retronasal OlfId testing is a low-cost, and quick screening which may be specifically useful when nasal polyposis are suspected.

123 Poster : Perception of Mixtures & Multimodal Stimuli

ODOR SUPPRESSION IN BINARY MIXTURES

Cashion L.[1], Livermore A.[2], Hummel T.[3] *[1]School of Social Sciences and Liberal Studies, Charles Sturt University, Bathurst, NSW, Australia; [2]Philip Morris USA, Richmond, VA; [3]Department of Otorhinolaryngology, University of Dresden Medical School, Dresden, Germany*

Previous research has suggested that odors that more strongly activate the trigeminal sensory system suppress those producing weaker trigeminal sensations and that mixed olfactory-trigeminal stimuli suppress odorants with little or no trigeminal activity. Following one weeks training, 20 normosmic participants were exposed to 6 odorants with varying trigeminal impact to test the hypothesis that stronger "trigeminal" odorants would suppress weaker trigeminal stimuli in binary odor mixtures. It was also hypothesized that stronger trigeminal odorants would dominate a 6-odor mixture. The predicted linear pattern of suppression was not seen, with a quadratic model emerging from the data. That is, "medium strength" trigeminal stimuli were found to have a stronger impact on odor detection than did weak and strong trigeminal stimuli. Similarly, although the linear and quadratic trends were not significant, medium strength trigeminal stimuli were better detected and perceived as more being more intense in the 6-odour mixture. It is proposed that the effect of suppression in binary mixtures was reliant upon at least two major effects, (1) the association formed between odours and the multiple memory systems that they interact with during the encoding and recognition processes, and (2) the balance between activation of the olfactory and trigeminal systems.

124 Poster : Perception of Mixtures & Multimodal Stimuli

EFFECTS OF PERCEIVED AND IMAGINED ODORS ON TASTE DETECTION

Djordjevic J.[1], Abrol K.[1], Boyle J.[1], Zatorre R.[1], Jones-Gotman M.[1]
[1]Montreal Neurological Institute, Montreal, Canada

We assessed the influence of two odors (strawberry and ham) on detection of a sweet tastant (sucrose), and the ability of imagined odors to elicit the same effects as perceived odors on taste perception. The aims of the study were to explore whether odor-induced effects on taste perception are a robust finding (i.e. could it be elicited by an objective measure), whether it is mediated by central or peripheral structures (i.e. could it be elicited by separate delivery of odorants and tastants), and whether imaging odors will yield the same results as smelling odors (if so, providing evidence of odor imagery). In the first experiment, participants (N=40) either smelled or imagined one of two odors during taste detection tasks (between-subject design), whereas in the second one, subjects (N=20) completed both the odor imagery and perception conditions (within-subject design). We found an odorant-specific effect in both experiments: detection of sucrose was significantly better when subjects smelled strawberry than when they smelled ham. Furthermore, in both experiments imagined odors influenced taste perception in the same way as did perceived odors. We concluded that odor-specific effects on taste perception are an authentic perceptual (and not a measure-specific) occurrence. Our results also support the notion that odor-induced changes of taste perception are a centrally mediated phenomenon. Finally, our findings provide further evidence for the existence of odor imagery. Observed individual differences in odor imagery ability were large, in keeping with our previous findings. Supported by Canadian Institutes of Health Research

125 Poster : Perception of Mixtures & Multimodal Stimuli

ODOR-INDUCED CHANGES IN TASTE PERCEPTION

Boyle J.[1], Djordjevic J.[1], Abrol K.[1], Perreau E.[1], Lakdawalla Z.[1], Zatorre R.[1], Jones-Gotman M.[1] *[1]Montreal Neurological Institute, Montreal, Canada*

The aim of this study was to examine whether odor-induced changes in taste perception are primarily a central or a peripheral phenomenon, whether this effect is a measurement artifact or not, and what is the direction of the effect (enhancement or interference). In the first experiment, participants (N=20) rated the intensity of taste-smell "mixtures", but with separate delivery of stimuli (nasal presentation of odorants and oral presentation of tastants). Perceived sweetness of sucrose was stronger with strawberry than with soy sauce odor, and perceived saltiness of sodium chloride tended to be stronger with soy sauce than with strawberry. In addition, results suggested enhancement of perceived taste intensity by a congruent odor. In the second (N=14) and third (N=20) experiments, participants detected peri-threshold taste stimuli while sniffing different odorants. Detection of peri-threshold sucrose was better with strawberry than with ham odor and worse with ham compared to a no-odor baseline, but there was no difference in detection accuracy comparing strawberry odor to baseline. In conclusion, we demonstrated odor-induced changes of taste perception using separate delivery of olfactory and gustatory stimuli, with both a subjective (intensity ratings) and an objective (taste detection accuracy) measure. Findings suggest that this is a centrally mediated, and a robust rather than a measure-specific phenomenon. Finally, the direction of the effect (enhancement or interference) may vary depending on procedural aspects of the experiment. Supported by Canadian Institutes of Health Research

126 Poster : Perception of Mixtures & Multimodal Stimuli

COLOR ENHANCES FRUITINESS

Zellner D.[1], Koza B.[1] [1]*Psychology, Montclair State University, Upper Montclair, NJ*

Colored solutions smell more intense than equally concentrated colorless ones (Zellner & Kautz, 1990). In those studies odors were identified to the participants prior to smelling. The intensity of the identified odor was rated. This study intestigated whether the effect occurs when participants are asked to rate the "fruitiness" of the odor without being told or knowing the identity of the odor. Fifteen participants smelled and rated the "fruitiness" of four different solutions presented in three successive random orders. Two of the solutions were Glaceau tangerine-pineapple-guava fruit water (one colored red and one colorless). The other two solutions were distilled water (one colored red and one colorless). Participants were blindfolded for the third set of ratings. Participants were not told the identity of the odor but were asked to guess its identity at the end of the experiment. No participant correctly identified the odor. The colored fruit water was rated as significantly more "fruity" than the colorless version $z=2.41$, $p=.016$. No such difference occurred when participants were blindfolded. Thus color-induced odor enhancement does not require knowledge of the identity of the odor.

127 Poster : Perception of Mixtures & Multimodal Stimuli

ODOR AND TASTE INTERACTION ON OLFACTORY EVOKED POTENTIALS

Kettenmann B.[1], Mueller C.[1], Wille C.[1], Kobal G.[1] [1]*University of Erlangen-Nuremberg, Erlangen, Germany*

Central neural integration of sensory input from different modalities is a prerequisite for flavor perception. In this study we interested the interaction of smell and taste information. Endpoints of the investigation were intensity estimates, hedonic ratings of the odors, and amplitudes and latencies of Olfactory Evoked Potentials (OEPs). Twelve subjects participated in the experiments. One out of three odorants (N-Butyl acetate, Linalool, Octanal) was applied in combination with five different tastants or blank (sweet, sour, salty, bitter, and umami). OEPs were recorded while the tastants were applied. Odorants had to be rated for intensity and hedonics after each of 16 stimuli. Odorants as well as tastants were applied in a randomized order. While Octanal was most intense in the presence of umami, N-Butyl acetate and Linalool showed no significant difference in any of the taste conditions. Octanal was rated more unpleasant than Linalool and N-Butyl acetate in all taste conditions. N-Butyl acetate became most pleasant in the presence of sweet taste. Linalool became most unpleasant in the presence of salty taste compared to blank. Apart from minor effects the N1-component after n-Butyl acetate stimulation was significantly smallest during sour, the P2-component after Linalool stimulation was significantly largest during bitter, and the P2-component after Octanal stimulation was significantly latest during salty taste. Interactions between odorants and tastants clearly were not uniform but followed specific and characteristic patterns which might have evolved due to learning processes. This research was sponsored by Procter & Gamble

128 Poster : Perception of Mixtures & Multimodal Stimuli

FLAVOR EXPECTATIONS

White T.L.[1], Prescott J.[2] [1]*Psychology, Le Moyne College, Syracuse, New York;* [2]*Sensory Science Research Centre, University of Otago, Dunedin, New Zealand*

The perception of flavor is formed cognitively from input originating from several sensory systems, the most salient of which is smell, followed by taste. The present set of experiments examine the degree to which olfactory information primes flavor expectations by readying the cognitive system to expect a particular type of taste. Previous evidence from a chemosensory Stroop-like reaction time task (White & Prescott, AChems 2001) has indicated that a discrepancy between previous experience and odor/taste pairs slows naming the tastant in the presence of the conflicting smell. The present experiments replicate these prior results by using additional odorants and extend them by asking whether the associations between smells and tastes taught to subjects would have the same priming effect that was observed in natural flavor pairings. Participants rated the sourness and sweetness of two odorants, then participated in a mock triangle test to provide them with extensive experience of a particular odor/taste combination. Odorant ratings were then repeated, followed by a taste identification task in which tastes were presented in the presence of odors that were either conflicted or associated. Shifts in ratings following the triangle test showed that odor/taste associations were formed. Reaction time measures indicated an effect of these learned associations on the speed of naming tastes, with congruent odors facilitating taste identification. The results of these experiments suggest that olfactory input primes the identity of a taste based on prior experience in the course of flavor perception. Supported by SOSI

129 Poster : Perception of Mixtures & Multimodal Stimuli

INTEGRATION OF TASTE AND ORALLY PRESENTED ODOR

Delwiche J.F.[1], Heffelfinger A.L.[1] [1]*FST Sensory Science Group, Ohio State University, Columbus, OH*

The anatomically distinct sensory apparatus for taste and smell, when stimulated simultaneously, often result in a unitary perception that seems to originate from the mouth. Ten subjects (6 m, 4 f; 21-24 yrs) assessed solutions of typical (sweet and pineapple) and atypical (monosodium glutamate (MSG) and pineapple) taste-smell pairs to investigate integration of taste and orally presented odor. Initially, the subject's taste threshold for sipping and spitting the solution was determined (using a signal detection technique) for both the aspartame/acesulfame K blend (sweet) and MSG. Control measures (20 2-AFCs while wearing a nose clip) indicated pineapple extract was tasteless at the concentrations used ($p>0.05$). In a subsequent session, odor thresholds were determined in the same manner, and then the subject assessed typical and atypical taste-odor pairs at one-half threshold concentrations. Odor-taste pairs were not distinguishable from pineapple solution by smell alone (20 2-AFCs while sniffing orthonasally; $p>0.05$), indicating no increase in odor of taste-odor pairs due to the addition of a taste compound. Using a signal detection measurement, the ability of subjects to differentiate between water and half-threshold taste-odor pairs was determined and results indicated that integration occurred between taste and orally presented odor compounds ($p=0.027$), regardless of typicality. Further, the results indicated that taste and orally presented odors showed complete additivity.

130 Poster : Perception of Mixtures & Multimodal Stimuli

IMAGES OF DESIRE: FMRI AND FOOD CRAVING

Pelchat M.L.[1], Ragland J.D.[2] [1]*Monell Chemical Senses Center, Philadelphia, PA;* [2]*Psychiatry, University of Pennsylvania, Philadelphia, PA*

Food craving (an intense desire to eat a specific food) is an extremely common, highly compelling psychological experience that has been linked to binge eating, snacking behavior, and maintenance of a varied diet. In addition, intense desire for foods may be the primal source for cravings of all kinds (e.g. for illicit drugs, sex, shopping). It is therefore surprising that so little is known about brain organization of food craving. We report here the first functional magnetic resonance imaging (fMRI) study to explicitly measure food craving (as opposed to hunger-induced desire). A two-part technique was used to produce the food cravings. First, the threshold was reduced through a diet manipulation (monotonous diet), without relying on hunger or other nutritional need. Second, cravings were triggered during the imaging sessions by having subjects imagine the sensory properties of favorite, but temporarily forbidden, foods (a cue-induction technique). Subjects (N = 20) were also asked to imagine the monotonous diet (which they did not crave). Signals generated while imagining the monotonous diet were subtracted from signals generated while imagining desired foods. This allowed us isolate craving-related signal changes while removing activation related to imagining food whether it is desired or not. Craving-related changes in blood oxygenation level dependent (BOLD) fMRI signal were identified in the hippocampus, caudate, and insula. The prominent representation of memory and sensory structures in the functional image is consistent with the sensory-specificity of food cravings (e.g. "It has to be chocolate ice cream, lemon pie won't do"). These results also support the hypothesis that there are common brain mechanisms for food and drug cravings.

131 Poster : Perception of Mixtures & Multimodal Stimuli

NEURAL CORRELATES OF CHEMOSENSORY INTEGRATION IN HUMANS STUDIED WITH FMRI.

Voss J.L.[1], Mak E.Y.[2], Simmons K.B.[2], Parrish T.B.[2], Small D.[2] [1]*Institute for Neuroscience, Northwestern University, Evanston, IL;* [2]*Cognitive Neurology and Alzheimer's Disease Center, Northwestern University, Chicago, IL*

Flavor perception involves the integration of peripherally distinct sensory input. We used fMRI to identify the network responsible for suprathreshold integration of taste and smell. A slow event-related fMRI design was performed on a Seimens Trio 3T magnet to measure activity in response to 5 solutions: 1) sucrose, 2) saline, 3) vanilla, 4) mixed sucrose and vanilla (congruent), and 5) mixed saline and vanilla (incongruent). Stimuli were delivered as 0.5ml boluses over a five second period. Subjects were cued to swallow by a tone and swallow-related movement was modeled out of the design. Intensity, familiarity and pleasantness ratings were collected at the beginning, middle and end of the scanning session. SPM99 was used for pre- and post-processing and small volume corrections were used to establish significance (p corrected <0.001). As predicted, greater activation was observed in the orbitofrontal cortex, anterior insula, and anterior cingulate cortices in response to the congruent mixture compared to 1) the summed activation of its independently presented constituents and 2) the incongruent mixture. These results are in accordance with previous electrophysiological and psychophysical evidence suggesting preferential central integration for previously experienced taste/smell combinations. Structural equation modeling is currently underway to determine if there is greater network coherence during congruent compared to incongruent presentations.

132 Poster : Perception of Mixtures & Multimodal Stimuli

BITTER-SWEET STIMULUS ANTAGONISM IN THE PERIPHERAL GUSTATORY SYSTEM

Formaker B.K.[1], Hettinger T.P.[1], Frank M.E.[1] [1]*Neurosciences, Dept. Oral Diagnosis, UConn Health Center, Farmington, CT*

Mixture combinations of quinine hydrochloride (QHCl) with sucrose or dulcin reduce hamster (*Mesocricetus auratus*) chorda tympani (CT) responses to the sweetener components; denatonium also reduces CT responses to sucrose (Formaker & Frank, 1996; Formaker, et al., 1997; 2001). To expand upon the hypothesis that aversive, bitter stimuli inhibit sweet stimulus responses, whole CT responses to mixtures of sucrose octaacetate (SOA) or KCl with sucrose, and single fiber CT responses to QHCl with sucrose or dulcin were measured. KCl and QHCl behaviorally cross-generalize (Frank & Nowlis, 1989) and SOA, although it does not cross-genralize with QHCl, is aversive to hamsters (Bouverat et al., 1997). Binary mixtures were prepared so concentrations in mixtures equaled component concentrations. Combinations of 0, 30, 100 and 300 mM sucrose with like concentrations of KCl and 1 mM SOA were used in the whole CT experiments; 0, 1, 3 and 10 mM QHCl were mixed with 100 mM sucrose and 5 mM dulcin in the single fiber experiments. Unlike QHCl, SOA did not inhibit 2.5-10 sec steady-state responses to sucrose. Subtraction of KCl response components from mixture responses revealed sucrose suppression that increased with KCl concentration. Sucrose suppression also increased with QHCl concentration in single fibers responsive to sucrose. Although the mechanisms involved may depend on the particular stimulus components, these results support a basic antagonism between bitter and sweet taste stimuli in the peripheral gustatory system. Supported by NIH: R01 DC 0409

133 Poster : Perception of Mixtures & Multimodal Stimuli

AMILORIDE BLOCKS NACL INHIBITION OF CT RESPONSES TO QUININE

Gordon P.E.[1], Frank M.E.[1], Formaker B.K.[1] [1]*Neurosciences, Dept. Oral Diagnosis, UConn Health Center, Farmington, CT*

We hypothesize that activation of taste-receptor cells by quinine·HCl is inhibited when the amiloride-sensitive Na^+ channel is active. In this study we compare interactions of 50 mM NaCl and 3 mM quinine·HCl in the presence and absence of 30 μM amiloride. Neural responses were recorded from chorda tympani nerves (CT) of 6 golden hamsters (*Mesocricetus auratus*) when NaCl, quinine·HCl and their mixture were applied to the tongue. Deionized water was used to rinse between stimuli. Responses to 500 mM NH_4Cl were used to normalize measurements, which were analyzed by ANOVA, $\alpha = .05$. Responses to the taste stimuli changed with amiloride treatment. With no amiloride, the NaCl-quinine mixture produced the same level of CT response as NaCl alone, less than the sum of individual responses, as reported previously (Formaker and Frank, 1996). When the tongue was treated with amiloride, the response to the NaCl-quinine mixture equaled the NaCl and quinine responses added together. We interpret this as follows. In the absence of amiloride, Na^+ enters the amiloride-sensitive channel, which may act to short-circuit the amiloride-insensitive pathway used by quinine·HCl, and the mixture response is nearly the same as the NaCl response alone. The response additivity with amiloride treatment suggests that blocking that channel eliminates the mixture interaction and supports this hypothesis. These findings are relevant to understanding the transduction mechanisms involved in the perception of salty and bitter salts. [Supported by NIH: T35 DE07136 & R01 DC04099]

134 Poster : Perception of Mixtures & Multimodal Stimuli

ANALYTIC NATURE OF TASTE MIXTURE INTERACTIONS: CONTRIBUTIONS OF PROP STATUS

Yiee J.H.[1], Duffy V.B.[2], Bartoshuk L.M.[3] [1]*Yale University, New Haven, CT;* [2]*Dietetics Program, University of Connecticut, Storrs, Connecticut;* [3]*Surgery, Yale University, New Haven, Connecticut*

In analytic mixtures, components maintain their identity (e.g., low and high notes sounded together). In synthetic mixtures, components lose their identity and new qualitative sensations appear (e.g., red and green lights produce yellow). Taste mixtures lose some components through suppression but new qualities do not appear. Prescott et al. (2001) showed that the degree of mixture suppression depends on the genetic ability to taste PROP. We extended his finding to three and four component mixtures and confirmed his conclusions. Subjects (N=65) rated the tastes of 0.32 M NaCl, 1 M sucrose, 0.014 M citric acid, 0.00024 M quinine hydrochloride, all six possible mixtures of two, all four possible mixtures of three and the single mixture of all stimuli. They also rated the tastes of foods/beverages (tonic water, lemonade, grapefruit juice, soy sauce, coffee sweetened with sucrose). Bitterness of PROP was rated at the end of the experiment. Subjects used the general Labeled Magnitude Scale (gLMS), which is the LMS developed by Green et al. (1993) with "strongest imaginable sensation of any kind" at the top. By labeling the scale in terms of all sensations, it provides valid across-group comparisons among nontasters, medium tasters and supertasters of PROP. PROP status affected suppression. Further, specific taste components in the foods/beverages varied with PROP status (e.g, the sweet taste in lemonade correlated with PROP, r=.40, p=.001). Of special relevance to the analytic/synthetic debate, the components that were significant in each mixture were those appropriate to the stimuli present with one exception: bitterness was suppressed as number of components went up, particularly for nontasters. Funding: DC00168.

135 Poster : Perception of Mixtures & Multimodal Stimuli

DISCRIMINABILITY OF TASTES WITHIN FOODS IN PROP TASTER GROUPS

Prescott J.[1], Soo J.[1] [1]*Sensory Science Research Centre, University of Otago, Dunedin, New Zealand*

Variations in sensitivity to the bitterness of 6-n-propylthiouracil (PROP) have also been associated with similar variations in sensitivity to other bitter compounds, as well as other taste qualities. It is presumed that such variations in perception underlie the differences found between PROP taster groups in preference for foods, in particular those that are bitter. To date, however, there have been few demonstrations that taste qualities within foods are also perceived differently by PROP taster groups. We compared groups of non-tasters (NTs), medium-tasters (MTs) and super-tasters (STs), defined using LMS ratings of 0.0032 M PROP, in their ability to discriminate variations in the sweetness (sucrose) and bitterness (caffeine) of orange juice, the sweetness (sucrose) and sourness (citric acid) of yoghurt, and the sourness (citric acid) and bitterness (caffeine) of cream cheese. In each case, samples consisted of commercial products to which the tastants were added. Forced choice tests were used to compare a reference sample with four other variations in tastant concentrations (usually 2 higher and 2 lower). For every set of comparisons, the JNDs and Weber ratios were smaller for the STs than for the NTs. Based on significance criteria for 2-AFC tests, STs showed significantly better discrimination at either higher or lower concentrations for bitterness in orange juice and cream cheese, sweetness in orange juice, and sourness in yoghurt and cream cheese. It is likely therefore that STs are more sensitive to variations in real foods than NTs.

136 Poster : Aging

BIOINFORMATICS ANALYSIS OF GENE EXPRESSION IN THE OLFACTORY-NASAL MUCOSAE OF SENESCENCE-ACCELERATED MICE

Getchell T.[1], Green C.P.[2], Peng X.[3], Stromberg A.[3], Chen K.[4], Mattson M.[5], Getchell M.L.[2] [1]*Physiology,* [2]*Anatomy and Neurobiology,* [3]*Statistics,* [4]*Molecular and Biochemical Pharmacology, University of Kentucky,* [5]*Laboratory of Neurosciences, NIA, Baltimore, MD*

High density oligonucleotide arrays were used to study gene expression profiles in the sensescence-accelerated mouse (SAM) as a biogerontological resource for studies on aging (TV Getchell et al., Ageing Research Reviews, 2003). Total RNA was isolated and pooled, three littermate mice per group, from olfactory-nasal mucosae of near-age matched old senescence-resistant (SAMR-O) and senescence-prone (SAMP-O) inbred mice. Following hybridization with Affymetrix GeneChips, three GeneChips per group, the signals were analyzed with GeneChip Analysis Suite v5.0 and statisitical software including Excel, S-PLUS6.1 and SAS. Pairwise comparisons were made between the experimental SAMP-O and the baseline SAMR-O arrays. The correlation coefficient between two SAMR-O or two SAMP-O samples was high, with an r value of 0.99, suggesting little variation due to tissue collection, total RNA isolation or hybridization procedures. In contrast, the r value between the SAMP-O and SAMR-O samples was 0.87, suggesting that signal variance could be due to age-related differences in the tissue samples. Of the 7941 known genes and ESTs 'Present' on at least one GeneChip, 715 known genes in the SAMP-O or the SAMR-O samples had a P value of < 0.05, suggesting a significant difference in their expression levels. A greater number of genes were downregulated (408) than upregulated (307). Categorization of gene expression profiles based on protein translation and cluster analysis further suggests functional groupings among known genes. Our results suggest that molecular phenotyping of expression profiles in the SAM mice will lead to further insight into genomic aging in the olfactory-nasal mucosae. NIH-AG-016824 (TVG).

137 Poster : Aging

AGE-RELATED APOTOSIS IN RAT OLFACTORY SENSORY NEURONS

Robinson A.M.[1], Conley D.B.[1], Kern R.C.[1] [1]*Otolaryngology-HNS, Northwestern University, Chicago, IL*

Apoptosis of olfactory receptor neurons (ORN) occurs at a baseline rate, likely as a response to environmental injury. ORN number is maintained by regeneration from precursors in the olfactory epithelium (OE). Data from both humans and rodents indicates that this process fails with age although the mechanism is unknown. Studies from our laboratory have demonstrated age-related increases at the RNA level for the pro-apoptotic proteins Bax and Procaspase-3 in the rat OE. In the current study, immunocytochemistry for active caspase-3, the primary executioner caspase for neuronal apoptosis, was utilized in F344xBN rats from 3 age groups (3, 18 and 30 months). In 3 month-old rats a small, randomly dispersed population of ORNs demonstrated caspase-3 activity, consistent with the baseline level of apoptosis observed in the OE. In the 18 month old middle-aged rats, an increased number of ORNs were positive for active caspase-3 and this increase was concentrated in the most ventrally located turbinates. In these turbinates the axon bundles within the underlying lamina propria demonstrated increased staining for active caspase-3. The 30 month-old rats demonstrated a further increase in the number of ORN cell bodies positive for active caspase-3. In these old animals active caspase-3 was now evident in the more dorsally situated turbinates, in addition to the more ventral turbinates. In conclusion, we found an age-related increase in the activation of caspase-3, suggesting an increase in the propensity of olfactory sensory neurons to undergo apoptosis in older animals. These results are consistent with the hypothesis that age-related olfactory loss is, at least in part, secondary to increased ORN cell death. R03 AG19965-01 (NIA).

138 Poster : Aging

AGE-RELATED CHANGES IN OLFACTORY PERFORMANCE : SENSORY, COGNITIVE AND RESPIRATORY FACTORS

Geisler T.[1], Hübener F.[1], Laska M.[2] *[1]Generation Research Program, University of Munich, Bad Tölz, Germany; [2]Department of Medical Psychology, University of Munich, Munich, Germany*

Two hundred healthy elderly subjects (60-94 years) and 20 young controls (25-34 years) participated in tests of olfactory sensitivity, discrimination ability and odor identification performance, as well as a screening test of global cognitive functioning, and tests of nasal and oral respiratory function. The results of the present study confirm earlier findings that aging is associated with a decline in all measures of olfactory performance. In addition, we found a) that age-related impairment of smell function is a process that starts well before the age of sixty and continues throughout the later stages of life, b) that different measures of olfactory function differ in their course and degree of age–related changes, and c) significant correlations between measures of olfactory function and measures of cognitive and respiratory function. Taken together, these results suggest that age-related changes in olfactory performance may not only be due to impairment of sensory function, but that impairment of cognitive and respiratory function may contribute to the decline of the sense of smell with advancing age. These findings may have important implications for the interpretation of impairments of smell function as a diagnostic marker for neurodegenerative diseases such as Alzheimer´s and Parkinson´s disease, and for training programs aiming at compensation for age-related loss of chemosensory function.

139 Poster : Aging

APOLIPOPROTEIN E4 POSITIVE INDIVIDUALS EXHIBIT GREATER DECLINE IN ODOR IDENTIFICATION THAN IN ODOR THRESHOLD OR DRS SCORES

Calhoun-Haney R.M.[1], Balie J.[1], Zizak V.[1], Ramage E.[1], Dulay M.[1], Murphy C.[2] *[1]Psychology, San Diego State University, San Diego, CA; [2]Psychology, San Diego State University, San Diego, California*

Deficits in olfactory function have been found in individuals with the apolipoprotein E e4 gene which is considered a genetic risk factor for developing AD. The present study investigated odor identification (odor ID), odor threshold, and global cognitive status in allele positive (E4+) and negative (E4 -) persons over a four year time span. Each participant was initially given the San Diego Odor Identification test, a butanol odor threshold test, and the Dementia Rating Scale (DRS), and were re-tested approximately four years later. The results suggest that odor ID declines more rapidly than either odor threshold or global cognitive status in normal elderly adults. In addition, E4 + individuals declined in odor ID with an effect size of eta2 = .76, and E4 – participants declined in odor ID with an effect size of eta2 = .41. The results indicate that the E4 gene may affect the semantic network of odors earlier than both odor detection ability or global cognitive status because participants exhibited a decline in odor ID but not odor threshold or DRS scores. Therefore, assessing olfactory function in conjunction with neuropsychological performance may facilitate early detection of AD, especially in those individuals at greater risk for developing the disease. Funded by NIH grant #AG04085.

140 Poster : Aging

OLFACTION ASSESSMENT IN CUBAN-AMERICAN ADULTS USING THE UNIVERSITY OF PENNSYLVANIA SMELL IDENTIFICATION TEST

Lee D.J.[1], Doty R.L.[2], Lam B.L.[3], Gomez-Marin O.[4], Jane D.[5] *[1]Epidemiology and Public Health, University of Miami School of Medicine, Miami, FL; [2]Smell and Taste Center, University of Pennsylvania, Philadelphia, PA; [3]Ophthalmology, University of Miami, Miami, FL; [4]Obstetrics & Gynecology, University of Miami School of Medicine, Miami, FL; [5]Epidemiology, University of Miami School of Medicine, Miami, FL*

In this study, we sought to determine whether older Cuban Americans scored similarly to their non-Hispanic counterparts on a 20-item version of the University of Pennsylvania Smell Identification Test (UPSIT). The test was administered to 50 community-residing Cuban Americans [mean (SD) age = 70.2 (10.5)] and compared to scores based on the same items obtained from 50 matched non-Hispanics from a database maintained at the University of Pennsylvania Smell and Taste Center. The mean number of correctly identified odorants did not differ significantly between the two groups, suggesting the utility of this 20-item test in assessing function in this largely Spanish-speaking population. As noted in other populations, the UPSIT scores were lower in males than in females, and in smokers than in non-smokers, and were inversely correlated with age. These data suggest that a 20-item version of the UPSIT may be of value in testing older members of the Cuban American population. Supported by grant # AG018996 from the National Institute on Aging

141 Poster : Aging

EFFECT OF ANTICHOLINERGIC MEDICATIONS ON TASTE, SMELL, AND OTHER SENSES AS WELL AS COGNITION IN THE ELDERLY

Schiffman S.S.[1], Kondor A.M.[1], Zervakis J.[1] *[1]Psychiatry, Duke University, Durham, North Carolina*

Anticholinergic medications have been reported clinically to impair both sensory perception and cognition but this has not been tested experimentally. This cross-sectional study examined the effect of anticholinergic medications taken systemically on taste and smell as well as vision, audition, somesthesis, and cognition. Two groups of healthy elderly persons aged 65-85 yrs were tested: 1) taking no medications other than hormone replacement (control group) and 2) taking at least one anticholinergic medication (experimental group). The battery of tests included: a) taste thresholds as well as a magnitude matching task for NaCl, sucrose and quinine HCl, b) smell detection threshold for butanol, c) smell identification, adaptation, and memory tasks, d) visual measures of near vision and far vision, e) hearing measures consisting of auditory thresholds at 6 frequencies, f) touch thresholds using domed gratings, g) measures of memory and attention. In addition, a medical background, self-rating of senses, and a measure of salivary flow were collected. Statistical analysis indicated that the medication group was significantly more likely to report a dry mouth and also tended to report more changes to vision and to taste. The medication group tended to perform more poorly than the control group on the Mini-Mental State Examination (MMSE) and quinine detection threshold. Further analyses revealed: a) performance on one paired associate task was correlated with smell identification and smell memory tasks, b) MMSE was correlated with paired associate tasks, digit symbols, and near vision measures, and c) salivary flow was correlated with quinine recognition thresholds. Supported by NIA AG 0443.

142 **Poster : Aging**

TASTE AND ODOR THRESHOLDS IN HEALTHY MIDDLE-AGED AND ELDERLY PARTICIPANTS OVER AN EIGHT YEAR TIME SPAN

Ramage E.[1], Calhoun-Haney R.M.[1], Zizak V.[2], Pirogovsky E.[2], Bailie J.[3], Espineli K.[2], Murphy C.[4] *[1]San Diego State University, San Diego, California; [2]San Diego State University, San Diego, CA; [3]San Diego State University, San Diego State University, CA; [4]Psychology, San Diego State University, San Diego, California*

Cross-sectional research suggests that significant olfactory impairment occurs as a result of aging. In addition, differences in taste thresholds for a variety of taste compounds exist between young and elderly participants. The question arises whether performance in taste and odor thresholds differ over time in older adults. The purpose of this study is to compare taste and odor thresholds over time (eight year interval) in participants 40 years and older. Taste (sucrose, caffeine, NaCl, citric acid) and odor threshold (butanol) thresholds for twenty-eight participants (M = 65, SD = 16)were tested on separate days in both 1993 and 2001. A repeated measures ANOVA revealed a main effect of visit, ($p<.005$), but none for gender. As expected, odor threshold significantly declined over time ($p<.008$). However, taste thresholds exhibited a practice effect for NaCl and caffeine ($p<.04$), a trend towards a practice effect for sucrose ($p< .10$), and no difference was found over time for citric acid ($p>.50$). These results suggest aging affects taste and odor systems differently and supports practice effects on taste threshold. Furthermore, these results highlight the benefit of practice for taste sensation in older adults. However, the olfactory system does not appear to share this advantage. Supported by NIH grant # AG04085 to C.M.

143 **Poster : Aging**

OLFACTORY AND TRIGEMINAL CHEMOSENSORY INTERACTION IN THE NORMOSMIC ELDERLY

Kurtz D.[1], Newlon J.[1] *[1]Neuroscience and Physiology, State University of New York Upstate Medical University, Syracuse, NY*

Our investigations of the effects of aging on the quality perception of odorants in normosmic subjects suggests that changes in the perception of odorants with significant nasal irritation may play a role in the alteration of perceived odorant quality in the normosmic elderly. A distinct interaction between odorant perception and irritant perception has previously been reported (Cain & Murphy, 1980) such that trigeminally mediated nasal irritation (pungency) suppresses the perceptual intensity of a simultaneously presented odorant. In the current study, we investigated the interaction of the common chemical sense and odorant perception in people who score well on tests of odorant identification (UPSIT $\geq$ 32). Young and elderly subjects were asked to rate the intensity perception of a single odorant (d-limonene) at each of four constant concentrations when mixed with various concentrations (0-38% v/v) of carbon dioxide using the labeled magnitude scale (LMS) (Green et al, 1993). In the elderly, the carbon dioxide had very little suppressive effect on the perceptual intensity of the odorant, while their younger counterparts demonstrated a high suppressive effect. Therefore, aging appears to result in both a suppression in the perceptual intensity of irritation (previously reported) and the loss of irritant induced reduction of odorant perceptual intensity. If these findings are extended to the perception of a single odorant containing both irritant and odorant qualities, it is possible that altered odorant quality perception in the elderly may be the result of a relative increase in the odorant portion and a diminution in the irritation portion of the odorant.

144 **Poster : Aging**

THE INFLUENCE OF AGING ON TRIGEMINAL CHEMORECEPTION IN THE NORMOSMIC ELDERLY

Newlon J.W.[1], Kurtz D.[1] *[1]Neuroscience and Physiology, State University of New York Upstate Medical University, Syracuse, NY*

Our investigations of the effects of aging on the quality perception of odorants in normosmic subjects suggest that a part of the observed change in quality perception may be due to perceptual changes in those odorants that also produce nasal irritation. Decrements in nasal irritation have been reported in the elderly by previous investigators (Stevens & Cain, 1986). However, it is not clear whether this effect of aging on the trigeminal system is concomitant to an olfactory loss or whether the aging of the two systems occurs separately. In the current study, we investigated the effect of aging on the common chemical sense in people who score well on measures of odorant identification. Suprathreshold irritation perceptual intensity was evaluated with the labeled magnitude scale at 26, 30, 34 and 38% v/v CO_2. Irritation threshold was evaluated with a two-interval forced choice staircase procedure. Elderly subjects (aged 65+) rated the suprathreshold irritation of CO_2 significantly less than young subjects (age 18-26). Additionally, nasal irritation threshold was significantly elevated in the elderly over the young group. We conclude that the aging process results in decrements in odorant-induced nasal irritation and elevation in the irritation threshold. Extending our previous work, we suggest that even in the relatively normosmic elderly, odorant quality perception is altered and that only part of that alteration is due to a decrement in the trigeminal component of odorant perception.

145 **Poster : Aging**

AGE-RELATED CHANGES IN BITTER AND SWEET SENSATIONS MAY INFLUENCE DIETARY BEHAVIORS

Chapo A.K.[1], Bartoshuk L.M.[1], Duffy V.B.[2] *[1]Surgery, Yale University, New Haven, CT; [2]Dietetics, University of Connecticut, Storrs, Connecticut*

This study explores relationships between oral sensation and diet in young (n=39, 25±4 y) and aged (n=41, 68±6 y) women, the latter postmenopausal and not taking estrogen replacement. Females used the general Labeled Magnitude Scale (gLMS) to rate intensities of 1M sucrose and 1 mM QHCl in solution as well as level of salty, sweet, sour, bitter, and creamy sensations in three sweet and two bitter foods, expressed as percent of total intensity. They also reported acceptance of bitter and sweet foods on the gLMS and frequency of consuming foods with these qualities. Young and aged did not differ significantly in taste genetic markers (PROP bitterness, fungiform papilla density). Young tasted greater sucrose and QHCl in solution. In sweet foods, young reported greater percentage of sweetness while aged reported greater percentage of creaminess. Aged reported more liking of high-fat, added and natural sweets but consumed low-fat sweets most frequently. Young preferred sweetened beverages and consumed these and desserts most frequently. For sampled bitter foods, young reported greater percentaged of bitter/sour sensations than aged; this matched acceptance and intake findings. Aged report greater liking and more frequent consumption of bitter foods. Summary: Supporting previous findings (Chapo et al, 2002), aged showed shifts from sweet to creamy sensations. Aged also showed declines in bitterness alone and in mixture. Aged preferred sweet and fat foods more but appeared to use restraint in consuming desserts. Age-related bitter declines may support diets rich in fruits and vegetables. (NIA RO3AG18619 funded)

146 Slide : Functional Organization of Gustatory Systems

STRUCTURE FUNCTION-RELATIONS OF BITTER TASTE RECEPTORS

Bernd B.[1], Hofmann T.[2], Krautwurst D.[1], Meyerhof W.[1] *[1]Molecular Genetics, German Institute of Human Nutrition, Bergholz-Rehbrücke, Germany; [2]Institute of Food Chemistry, University Münster, Münster, Germany*

Increasing the content of chemoprotective compounds in plant food is a potent dietary option for the prevention of diseases and a challenge for the design of novel food. However, the consumer rejects food rich in these bitter tasting phytonutrients thereby promoting their removal during breeding and food processing. The development and employment of bitter masking agents could circumvent this problem, but require a detailed understanding of the interaction of bitter compounds with their receptors on the human tongue, which, however, has not been achieved yet. In an attempt to identify bitter receptors we cloned all human and serveral rodent members of the *TAS2R* gene family. Using functional expression of the *TAS2R* genes in HEK293/Gα15 cells and calcium imaging detection we identified several cognate-ligand-receptor-pairs. Detailed analysis of *hTAS2R16* revealed that this receptor is activated by bitter pyranosides and allowed us to define structural parameters necessary for receptor-agonist interactions. Another human receptor, *hTAS2R10*, responds to strychnine, while its closest rodent relatives *rTAS2R9* and *mTAS2R5* respond to cycloheximide. Therefore, these receptors are ideal candidates to study the structural requirements necessary for receptor activation. Sequence comparison, EC_{50} derived tastant profiles and receptor modeling allow us to determine amino acid residues important for receptor activation.

147 Slide : Functional Organization of Gustatory Systems

KNOCKOUT MICE FOR TASTE SIGNAL TRANSDUCTION STUDIES

Rong M.[1], He W.[1], Yasumatsu K.[2], Ninomiya Y.[2], Margolskee R.F.[3], Damak S.[1] *[1]Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY; [2]Section of Oral Neuroscience, Graduate School of Dental Sciences, Kyushu University, Fukuoka, Japan; [3]Howard Hughes Medical Institute, Mount Sinai School of Medicine*

We have identified several components of the signaling pathways involved in transducing responses to sweet, bitter and/or umami compounds. These include: 1) α-gustducin, a G protein α-subunit, 2) Gγ13, the γ-subunit of gustducin, 3) α-transducin, another taste cell-expressed G protein α-subunit, 4) Trpm5, a novel store activated calcium channel expressed selectively in taste receptor cells. Knockout mice provide a powerful tool for elucidating the function of novel gene products in vivo. In addition to the α-gustducin knockouts, which demonstrated the key role that this α subunit plays in bitter and sweet signal transduction, we have produced knockout mice for α-transducin and Trpm5. We are also generating knockouts for Gγ13 and other taste transduction elements. α-Transducin/α-gustducin double knockout mice were generated by crossing the single knockouts over two generations. While the α-gustducin KO mice showed reduced preference for concentrations of MSG that were highly preferred by wild type (WT) mice, the double knockouts displayed no preference at all for MSG. α-Transducin knockouts did not differ from WT mice in their behavioral response to MSG. Thus the behavioral data indicate that both G protein α subunits play a role in the transduction of MSG taste signals and that α-gustducin plays a predominant role. Preliminary nerve recordings from these mice yielded results consistent with the behavioral data. Trpm5 KO mice are viable and healthy, and are currently being used for behavioral and electrophysiological studies. Preliminary results with these mice have confirmed their importance to taste transduction.

148 Slide : Functional Organization of Gustatory Systems

FUNCTIONAL INTERACTIONS BETWEEN TASTE PAPILLAE: A SINGLE UNIT STUDY IN THE HAMSTER

Vandenbeuch A.[1], Pillias A.[1], Faurion A.[1] *[1]Lab Neurobiologie Sensorielle, Massy, CEDEX, France*

A modulation of chorda tympani nerve (CT) single fibre responses was formerly demonstrated by stimulating a single papilla and its surrounding receptive field (Miller, 1971). In the present study, aiming at exploring the complex underlying neural network, single taste fibres were recorded through fungiform papillae taste pores while either one or two neighbouring single papillae were individually stimulated. The intrapapillar recording technique (Faurion and Courchay, 1990), using 20 µm o.d. 0.9% NaCl filled glass micropipettes was associated to iontophoretic stimulation of single papillae using 20-30 µm o.d. pipettes either filled with 100 mM acesulfam K, 100 mM monosodium L-glutamate or 155 mM NaCl. Out of 304 papillae stimulated with either of these ions, 40 elicited single unit responses (30 activations, 10 inhibitions) in a papilla recorded nearby. In 54 cases, two papillae were simultaneously stimulated with two independent micropipettes: 12 cases showed an inhibition of the response elicited compared to the response obtained for only one of these stimulations. The observation of decrementing spikes and the firing frequency reduction during double stimulations suggest that the activity of branched taste fibres could be modulated by local axo-axonic synapses, as the section of trigeminal and CT nerves did not result in any change in these response patterns. Further work using pharmacological agents is in progress to investigate the nature of such putative synapses within the lingual neural plexus. Supported by IGTC and EPHE to AF.

149 Slide : Functional Organization of Gustatory Systems

ORGANIZATION AND PLASTICITY OF GUSTATORY NERVE TERMINAL FIELDS REVEALED BY TRIPLE FLUORESCENT LABELING

May O.L.[1], Hill D.L.[1] *[1]Psychology, University of Virginia, Charlottesville, VA*

The chorda tympani (CT) and greater superficial petrosal (GSP) nerves terminate in the rostral most portion of the nucleus of the solitary tract (NTS). The glossopharyngeal (IX) nerve terminates caudal to CT and GSP fields. Previous research has documented the dorsal most portion of the CT field to be plastic in response to developmental dietary sodium restriction. However, this dietary manipulation does not apparently affect GSP or IX field development. In order to examine the relative relationships among CT, GSP, and IX terminal fields in adult control and developmentally sodium-restricted rats, we used triple fluorescence labeling in live rats. In control rats, GSP terminations begin more dorsal than and extend more ventral than the CT. IX terminations begin more dorsal than and extend ventrally into the dorsal most zone of the GSP. The overlap between GSP and IX fields is much greater compared to the CT and IX. CT and GSP terminations overlap completely within the rostral pole of the NTS. Sodium-restricted rats had an enlarged CT field that extended caudally into the GSP field. Unexpectedly, the IX field in sodium-restricted rats was expanded by 2-fold, extending ventrally through the CT field. The GSP field remained unchanged by the dietary manipulation. Thus, the degree of overlap among the three fields was more pronounced in restricted rats. These anatomical findings have clarified the relative relationship among primary afferents in the NTS and strongly suggest that there are corresponding convergent-related functional and behavioral alterations. Supported by NIH grant DC00407

150 Slide : Functional Organization of Gustatory Systems

DIFFERENTIAL RESPONSE TO FAMILIAR AND UNFAMILIAR TASTE IN THE GUSTATORY CORTEX OF THE FREELY BEHAVING RAT

Bahar A.[1], Dudai Y.[1], Ahissar E.[1] *[1]Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel*

Ample data suggest that the gustatory cortex (GC) plays an important role in the neural processing of unfamiliar tastes. To investigate the neural correlates of gustatory familiarity encoding, we employ chronically implanted multi-wire electrodes that record the activity of multi-units in the GC of rats licking unfamiliar or familiar solutions. We find that most recorded units show a typical lingering response (7s) to 1s of licking. A spike count analysis show that while the percentage of taste responding units does not change significantly between unfamiliar vs. familiar stimuli, the average spike count for taste vs. water of the entire recorded population increases significantly as taste becomes familiar, but only during the last 5s of the response. We find that the change is not correlated to familiarity detection per se, but to the familiarity process to a specific experienced taste. We identify 2 sub-groups in the population that undergo this change: units that increase their response to taste only in unfamiliar-familiar transition (68%), and units that continue to increase their response in correlation to increased familiarity in a third exposure. Our data imply that the GC responds differentially to familiar vs. unfamiliar taste during a distinct phase of the neural response, and corroborate the notion that multiple taste attributes, familiarity included, are encoded in relatively large groups of GC multi-units. (Supported by the Minerva stiftung and the Human Frontiers Science Program Organization (Y.D.), and by the Irving B. Harris and the Edith C. Blum Foundations (E.A.).

151 Slide : Functional Organization of Gustatory Systems

TASTE INFORMATION IN THE PRIMATE ORBITOFRONTAL CORTEX.

Verhagen J.V.[1], Rolls E.T.[1], Critchley H.[1], Kadohisa M.[1] *[1]Experimental Psychology, University of Oxford, Oxford, United Kingdom*

The macaque orbitofrontal cortex (OFC) contains the secondary taste cortex with taste-responsive neurons. Information theoretic analyses (Rolls et al, 1996, J Neurophysiol 75: 1982-1996) were used to investigate the nature of their representations. Responses to 0.2 ml of 1.0 M glucose, 0.1 M NaCl, 0.01 M HCl, 0.001 M quinine HCl, and 0.1 M MSG of 135 OFC taste cells were analyzed in a time period of 1 sec starting 100 ms after stimulus delivery. The average mutual information about which tastant was presented was large being 0.45 bits (sd=0.26) across the population of neurons. This is five times higher than previously reported for olfactory responses in OFC neurons for a set of 7-9 odors. The robustness of the encoding of taste stimuli reflects reliably different responses on a trial by trial basis to the different tastants. The stimulus-specific information about glucose was higher on average across the cells (0.47 bits) than for other tastants (0.26 bits). This particularly robust encoding of glucose is consistent with a high proportion of cells with best response to glucose (36%). Information analysis also showed that the amount of information encoded by a neuron about a particular tastant was related to the z-score of the response to a tastant. The stimulus-specific information about tannic acid was on average 0.9 bits across 7 tannic acid-best neurons (of 79 tested cells), and these neurons encoded less information about taste. The analyses show not only that there is a distributed encoding of taste in the OFC, but also that the encoding is reliable. Consistent with the distributed representation, the mean sparseness was 0.84 (sd=0.14).

152 Slide : Functional Organization of Gustatory Systems

DISSOCIATION OF HUMAN BRAIN REGIONS RESPONDING TO TASTE INTENSITY VERSUS TASTE AFFECT USING FUNCTIONAL MAGNETIC RESONANCE IMAGING

Gregory M.D.[1], Mak E.Y.[1], Parrish T.B.[1], Small D.[1] *[1]Cognitive Neurology and Alzheimer's Disease Center, Northwestern University, Chicago, IL*

The neural substrates of gustation must code for three perceptual dimensions: Intensity, affect and quality. To isolate brain regions responsive to taste intensity versus affect, we performed functional magnetic resonance imaging(fMRI) on healthy young subjects as they tasted two concentrations each of a pleasant and unpleasant taste. Nine subjects participated in a psychophysical evaluation followed by an fMRI study. Prior to scanning each subject rated sweet and bitter solutions for intensity and pleasantness on a 0-10 point scale. Stimuli concentrations were yoked so affect and intensity ratings were matched (Pleasant Strong: 8±1 for intensity and pleasantness; Pleasant Weak: 2±1 for intensity and 8±1 for affect; Unpleasant Strong: 8±1 for intensity and 2±1 for affect; Unpleasant Weak: 2±1 for intensity and affect). Seven 5:12 slow event-related fMRI runs were conducted on a Siemens 1.5 Tesla magnet. The four tastes plus a tasteless solution were presented twice per run as 5cc boluses. Group random effects analyses show that brain areas responsive to intensity irrespective of affective valence include the pons, dorsal anterior cingulate, middle ventral insula and amygdala. Areas responsive to affect regardless of intensity include the ventral anterior cingulate, anterior ventral insula and caudal orbitofrontal cortex. These results provide evidence that there is a functional dissociation of regions coding for taste affect versus taste intensity.

153 Slide : Functional Organization of Gustatory Systems

GUSTATORY CODING OF TASTE INTENSITY IN THE HUMAN AMYGDALOID COMPLEX

Small D.[1], Gregory M.[1], Parrish T.B.[2] *[1]Neurology, Northwestern University Medical School, Chicago, Illinois; [2]Radiology, Northwestern University Medical School, Chicago, Illinois*

Neuroimaging studies have shown that the amgydaloid complex (AC) is activated in response to aversive tastes, odors and flavors. However, aversive stimuli tend to be perceived as more intense than neutral or pleasant stimuli, and evidence is mounting to suggest that the AC is concerned with coding intensity rather than, or in addition to, affective properties of sensory stimuli. For example, patient studies show changes in taste intensity perception following resection of the AC for the treatment of epilepsy. Additionally, Sobel and colleagues have presented neuroimaging results showing that activity in the AC is related to intensity and NOT affective processing of odors. We used a similar design to evaluate AC response to taste intensity perception regardless of affect. Two concentrations of sucrose (pleasant) and quinine (unpleasant) solutions were presented. Stimuli were carefully equated for intensity and presented to 11 subjects in an event-related fMRI design, using a Seimens 1.5T magnet. Data were pre and post-processed with SPM99. A group random effects analysis revealed AC activation in response to intensity regardless of affective valence. These results provide further evidence that the AC is concerned with coding intensity rather than affective valence, Funding: NIDCD

154 Symposium : Interplay of Olfaction & Emotion Systems

THE ANATOMY OF EMOTION IN OLFACTION

Kay L.M.[1] [1]*Institute of Mind and Biology, University of Chicago, Chicago, IL*

The reciprocal relationship between olfaction and emotion is not a new concept. From the time of Proust and as far back as the Jewish mystics, it has been remarked that odors can rekindle long-buried memories and even uncover one's true nature. The neural mechanism more recently proposed for the preferential relationship between olfaction, memory and emotion, is the olfactory's system's privileged access to the limbic system. While other sensory signals pass through many subcortical and cortical areas before reaching the limbic areas, olfactory signals are two synapses away. All parts of the olfactory system also receive significant input from the amygdala and the hippocampal formation. This system is very stable across mammalian species. Connections present in rodents are almost identical in non-human primates and humans, particularly those between the olfactory bulb and the amygdala and hippocampus, where other sensory connections are in some cases severely reduced. Work from several laboratories now suggests that olfactory, amygdalar and hippocampal areas are so interconnected as to be considered one large system. Anatomical results uncover the detail of centrifugal input to the olfactory bulb, which provides a strong influence on sensory processing. These connections likely produce the context-dependent representation of odor stimuli by mitral cells in the olfactory bulb. Other studies show that odors with particular emotional significance (predators, poisons and potential mates) produce unique signals in olfactory and limbic brain areas. Thus, combined anatomical, physiological and behavioral approaches can now begin to unravel the complex relationships between emotion and olfaction.

155 Symposium : Interplay of Olfaction & Emotion Systems

OLFACTION: A PLAYGROUND FOR SENSATION, EMOTION AND COGNITION

Dalton P.[1] [1]*Monell Chemical Senses Center, Philadelphia, PA*

Even moderate fluctuations in affect can systematically affect higher-level cognitive processes involved in perception, interpretation, memory and judgment. It is therefore not surprising that the hedonic salience of many odorous stimuli can significantly mediate autonomic and cognitive processes via emotional arousal. Cognitive psychology provides a useful context for describing the sources of variation in responses to odors by describing these interactions in the framework of information-processing models. From this perspective, the emotional responses elicited by olfactory stimuli can be viewed as both an outcome of sensory stimulation and as modulators of cognitive and motivational processes. Interactions between sensory properties of an odorant (such as intensity or quality), states of emotional activation, and types of cognitive interpretations result in the production of different integrated perceptual experiences, each with marked influences on behavior.

156 Symposium : Interplay of Olfaction & Emotion Systems

LESSONS FROM THE EMOTION LAB

Haviland-Jones J.[1] [1]*Psychology, Rutgers University, Piscataway, NJ*

Over the last 25 years, theories and methodologies have changed tremendously in research on emotion. For behavior, film and video along with the sequential and dynamic analyses have been central. Integration of convergent methods of analyzing movement, social patterns and self-reports has led to better strategies of interpretation across levels of emotional responses. Emotion processes are "Smart" systems in the sense used by computer architects -- they are customized (adaptive) for specific tasks or users. The user seldom knows about the architecture of the system and is unaware of rapidity of processing making it largely unconscious. Even when part of the system – the signaling system, for example -- is general purpose (like a word processor), there are many ways to achieve the same ends. Methods of behavioral study are often very labor intensive but some involve "short cut keys" relying on sets of secondary cognitive and social behaviors. Take a quick example: Fresh, fragrant flowers affect positive emotion. Within 3 secs they elicit the Duchenne smile; in 4 minutes they change social proximity; in 3 days they change reports of daily mood; in two weeks they change memory/attention function. These are convergent indices of underlying positive emotion, seldom reported directly. Chemical signals, even ones produced internally, have such convergent effects on emotion behavior. Research is underway to clarify methods for testing, as the results of several studies will show. The system we expect to find is a dynamic one. To illustrate a working computer model will be presented.

157 Symposium : Interplay of Olfaction & Emotion Systems

EMOTIONAL ODORS AND THE FINAL COMMON PATH

Lorig T.S.[1] [1]*Psychology, Washington& Lee University, Lexington, VA*

Advances in neuroimaging have provided new and exciting knowledge concerning how odors come to activate emotional systems in the brain. Often neglected are the concomitant changes that follow this activation throughout the body. Odor induced emotional changes in peripheral physiological systems will be critically discussed including changes in respiration, muscle tone, skin conductance and heart rate. Multidimensional patterning of these responses may prove especially valuable in identifying subtle emotional responses.

158 Symposium : Interplay of Olfaction & Emotion Systems

NEUROIMAGING THE DYNAMIC INTERPLAY OF EMOTION AND OLFACTION

Zald D.H.[1] [1]*Dept of Psychology, Vanderbilt University, Nashville, TN*

Over the last decade, positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have helped to elucidate the distributed cortical and subcortical networks involved in olfaction and emotion. The study of olfaction and emotion in the neuroimaging environment poses unique methodological challenges. Nevertheless, this area of research has provided numerous insights into the dynamic and varied network of structures that are engaged by odorants. I will particularly focus on the extent to which factors such as emotional valence, emotional context, and explicit processes such as hedonic judgments influence activity within these networks.

159 Symposium : Interplay of Olfaction & Emotion Systems

EMOTIONAL OLFACTORY STIMULI: FROM UNCONSCIOUS TO CONSCIOUS PROCESSING

McClintock M.K.[1] [1]*Psychology, University of Chicago, Chicago, IL*

Olfactory chemosignals function at different levels of consciousness to modulate human emotional and social experiences. Some compounds are consciously detected as odors or scents, eliciting a variety of emotions and associations, even when their human origin is not recognized. Others are pheromones, operating in minute quantities and eliciting specific neuroendocrine responses or modulating behavior. Intermediate are a class of compounds that modulate emotional states without being detected verbally as an odor or a scent, yet are not pheromones because they are not social, do not operate in minute concentrations, or are not unique compounds with specific effects. To call them "unconscious odors" is not accurate, because odors are, by definition, consciously detected. To deal with this phenomenon of different levels of consciousness, we propose the term "vasana" for olfactory chemosignals in this intermediate class that affect human emotions yet are not available for direct conscious scrutiny. We anticipate that different brain mechanisms process the different classes of olfactory chemosignals. Specific examples will be discussed including the effect of MHC gene matching on emotional reaction to human compounds, steroids found in human sweat (androstadienone and estratetraenol), and the effect of human compounds on fertility and sexual desire.

160 Poster : Animal Behavior: Tracking and Orientation

THE CONSEQUENCES OF SPERM CHEMOATTRACTION FOR FERTILIZATION SUCCESS

Zimmer R.K.[1], Riffell J.A.[1], Krug P.J.[1] [1]*Department of Biology, University of California, Los Angeles, Los Angeles, California*

Chemical communication between sperm and egg is a critical factor mediating sexual reproduction. Sperm attractants may be significant evolutionarily for maintaining species barriers, and important ecologically for increasing gamete encounters. Yet, the ultimate consequences of these dissolved signal molecules have never been verified experimentally. Here, we provide the first evidence that sperm chemoattraction directly affects the magnitude of fertilization success. Our recent discovery of L-tryptophan as a potent attractant to abalone (Haliotis rufescens) sperm offered the rare opportunity to quantify how navigation affects gamete encounter rates. Sperm behavioral responses to manipulations of the natural tryptophan gradient around individual eggs reveal that both chemotactic and chemokinetic effects significantly enhance fertilization. Extending well beyond the reach of membrane-bound proteins, our results show that upstream soluble egg factors not only attract sperm, but play a direct role in expediting the sexual reproductive process.

161 Poster : Animal Behavior: Tracking and Orientation

PRE- AND POST-ODOR FLICKING BEHAVIOR IN THE CRAYFISH, *ORCONECTES RUSTICUS*, MEASURED AT DIFFERENT FLOW SPEEDS

Kraus-Epley K.E.[1], Moore P.A.[1] [1]*Biological Sciences, Bowling Green State University, Bowling Green, OH*

It has long been assumed that bilateral sampling through the flicking behavior of decapod crustaceans is essential for olfaction and that the flick is a stereotyped behavior, but no study has definitively demonstrated this latter fact to be true. In the current study, we sought to demonstrate that the flicking behavior of the crayfish, *Orconectes rusticus*, is a stereotypical olfactory-related phenomenon. Male crayfish were restrained and placed in a small recirculating flume and allowed to acclimate before the trial. Flicking was videotaped for 45 seconds before the onset of the odor (fish homogenate) and for 45 seconds post-onset of the odor. Animals were tested at two flow speeds: 1 cm/s and 3 cm/s. Prior to the acclimation period, points of reflective paint were positioned along the lateral antennules and on the rostrum to aid in the task of digitization. Peak Motus Motion Analysis System was used to digitize all points. Parameters analyzed were the following: pre- and post-odor flick rate, right to left antennule flick ratio, flick angle, and flick velocity. This study clearly shows that flick behavior changes with the onset of an odor source, that flicking also varies with relation to flow speed of the medium, and that these animals do not flick bilaterally. This research was funded by NSF #IBN-0131320.

162 Poster : Animal Behavior: Tracking and Orientation

IDENTIFICATION OF CHEMOSENSORY SENSILLA ACTIVATING ANTENNULAR FLICK BEHAVIOR IN THE CARIBBEAN SPINY LOBSTER, *PANULIRUS ARGUS*

Fox M.[1], Mehta S.[2], Daniel P.C.[3] [1]*John F. Kennedy High School, Bellmore, NY;* [2]*The Wheatley School, Old Westbury, NY;* [3]*Hofstra University, Hempstead, New York*

Lobsters sample odorants in the environment by a rapid series of flicks of the two flagella comprising the distal segment of the antennules. The lateral flagellum contains large numbers of aesthetasc sensilla, the only sensory neurons dedicated exclusively to detecting odorants. Other chemomechanosensilla are distributed on both lateral and medial flagella. In this study we identify the chemosensory sensilla that activate antennular flick behavior in *Panulirus argus* using either distilled water (DW) ablation or excision of specific setae. Flick rates towards squid extract were measured after each ablation. DW ablation of the lateral flagella or excision of the aesthetascs resulted in complete loss of responsiveness. Excision of guard and companion setae, putative sensilla also found on the lateral flagellum, had no effect on flick rates. DW ablation of the medial flagellum resulted in a 50% drop in flick rate. Thus normal flicking behavior requires both aesthetasc and non-aesthetasc sensilla. Results of ablation studies examining chemosensory mediation of other behaviors indicate that 1) aesthetascs are necessary and sufficient for chemosensory activation of antennular grooming behavior (Wroblewska et al., Chem. Senses 27:769-778) and 2) sufficient search behavior can occur in the absence of either aesthetascs or non-aesthetasc sensilla but not both (Steullet et al., J. Exp. Biol. 204:4259-4269). We propose that behaviors involving some degree of orientation (antennular flick and search) are driven by chemosensory input from both aesthetasc and non-aesthetasc sensilla.

163 Poster : Animal Behavior: Tracking and Orientation

CRAYFISH RESPONSE TO COMPLEX ODOR SIGNALS

Wolf M.[1], Voigt R.[1], Moore P.A.[1] *[1]Biological Sciences, Bowling Green State University, Bowling Green, OH*

Organisms in natural environments encounter complex information and must be able to decipher this information in order to respond behaviorally. This complex information can be a result of various aspects of the natural environment such as hydrodynamics, changing habitats, conflicting and multiple odor cues. Though there have been many studies investigating orientation mechanisms of aquatic organisms it is unclear is some cases the mechanisms that are guiding this behavior. The present study was designed to try and elucidate aspects of signal complexity that drive spatial and temporal aspects of orientation behavior. We used the crayfish O. virilis in orientation trials where they were presented with a series of odor source arrangements. Characterization of the odor plume structure and hydrodynamics of the stream were performed using IVEC-10 and acoustic Doppler velocimeter (ADV) systems. Crayfish significantly altered their orientation strategies in the presence differing spatial arrangements of food cues. Since the change in odor placement did not alter the hydrodynamic structure, changes seen in orientation behavior may indicate that changes in the fine scale structure of the signal are guiding behavior.

164 Poster : Animal Behavior: Tracking and Orientation

DISTINCTION BETWEEN THE ORIENTATION MECHANISMS OF RHEOTAXIS AND CHEMOTAXIS IN THE CRAYFISH THROUGH A BEHAVIORALLY-SELECTIVE LESION

Ramsey S.[1], Kraus-Epley K.E.[1], Moore P.A.[1] *[1]Biological Sciences, Bowling Green State University, Bowling Green, Ohio*

The goal of this project was to determine whether crayfish are orienting using a mechanism of chemotaxis or odor-gated rheotaxis. A true chemotaxis is orientation mediated purely 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 medium, using the chemoreceptors and mechanoreceptors, respectively. By selectively lesioning only chemoreceptors and not mechanoreceptors, we can determine whether these animals, are orienting using chemotaxis or odor-gated rheotaxis. Chemoreceptors on crayfish were lesioned by dipping their olfactory appendages in 50 ppt saltwater for two hours followed by deionized water for 10 minutes. The animals were subsequently 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 gives us insight into which orientation mechanism crayfish may be using to orient.

165 Poster : Animal Behavior: Tracking and Orientation

CHEMICALLY MEDIATED SEARCH BEHAVIOR OF GOLDFISH TO FOOD AND PHEROMONAL ODORS

Sherman M.L.[1], Sorensen P.W.[1] *[1]Fisheries, Wildlife, and Conservation Biology, University of Minnesota, Saint Paul, MN*

Although chemical cues are known to play a critical role mediating feeding and reproduction in fishes, how social species such as the goldfish actually employ odor and other cues to locate food and mates is poorly understood. Our study addressed this question by tracking the distribution of either individual or groups of male goldfish in a 2-choice laminar-flow maze to which a food odor or a spawning pheromone was added via a point source. Single goldfish located the origin of food and pheromone odors within 15 min, but were twice as successful at locating the food odor suggesting these cues stimulate different types of behavior. When tested as groups, goldfish located both odors with even greater success than they had as single fish with the increase being especially notable for food odor, which was located 10 times more quickly. While group responses to food odor were associated with long-term attraction and repeated sampling at the odor source, exposure to the sex pheromone stimulated only transient attraction and large increases in social behavior. In summary, goldfish are able to locate food and pheromone odors using concentration gradients alone but appear much better at doing so for food odor. Additionally, chemo-orientation toward both odor cues appears to be strongly facilitated by different types of social interaction presumably associated with visual cues. The tracks taken by single and grouped goldfish are now being analyzed to determine the specific orientation mechanisms underlying these responses. Supported by the Minnesota Department of Natural Resources.

166 Poster : Olfactory Transduction

ELECTROPHYSIOLOGICAL INVESTIGATION AND EFFECT OF FAST RELEASE OF CAGED MOLECULES ON OLFACTORY SENSORY NEURONS ISOLATED FROM MICE

Lagostena L.[1], Menini A.[1] *[1]SISSA, Trieste, Italy*

While many electrophysiological studies have been performed on olfactory sensory neurons (OSNs) from various species of vertebrates, data from rodents, especially from mice, are still lacking. This is due to the extremely reduced dimensions and increased fragility of mouse OSNs. On the other hand, gene manipulation techniques are well developed for mice. Therefore, successful isolation procedure for mouse OSNs is of increased importance for joining molecular biological and electrophysiological study on these cells. Some elegant investigations were conducted on mouse OSNs in the intact epithelium (in perforated patch-configuration) and/or on isolated cells with the suction technique. These approaches, while of great utility, present the disadvantage of an inaccessible intracellular environment. Because of this, controlling or introducing molecules to OSNs cytoplasm is impossible. Here we present electrophysiological characterization of and the effect of UV-photolysis of caged compounds on OSNs, isolated from mice in the whole-cell configuration. At least three voltage-gated currents (an inward and two outward currents) were identified. Loading the cytoplasm with different caged compounds, the effect of fast increase of these substances was investigated by UV-flash photolysis, allowing us to highlight intracellular processes and to resolve them in time and space. This approach opens a new field of investigation that, with the help of molecular biology techniques (such as the development of transgenic mice and gene targeting), can be useful to further our understanding of odor transduction in mice. Supported by NATO LST.CLG.978303

167 Poster : Olfactory Transduction

LIVE CELL IMAGING OF CA2+ DURING THE CHEMOATTRACTANT OFF-RESPONSE IN *PARAMECIUM*

Bell W.E.[1], Green M.H.[1], Van Houten J.[2] [1]*Biology, Virginia Military Institute, Lexington, VA;* [2]*Biology, University of Vermont, Burlington, VT*

Paramecium tetraurelia are unicellular eukaryotes with excitable membranes, which give them the capability to respond to differing environmental stimuli by altering membrane potential, and thus changing their swimming speed and direction. Among the ion channels involved in altering membrane potential are both ciliary and somatic Ca^{2+} channels. Somatic Ca^{2+} channels have been implicated in regulation of Na^{+}, K^{+}, and Mg^{2+} channels while ciliary Ca^{2+} channels are responsible for ciliary reversal. 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 calcium-sensitive dye Calcium Orange AM (Molecular Probes) were perfused with experimental attractants and control solutions and intracellular Ca^{2+} levels were observed. After removal of the attractant acetate from the bathing solution we observed a slow, but significant rise in intracellular Ca^{2+}. The Ca^{2+} increase returned to baseline without restimulation. Removal of the attractant ammonium chloride, an attractant that uses a unique signaling pathway different from acetate, did not result in a measurable Ca^{2+} influx. Since the removal of either attractant would result in ciliary reversal, we hypothesize that the Ca^{2+} influx we observe is entering through somatic Ca^{2+} channels. Now that we have the capability to observe specific attractant-mediated Ca^{2+} fluxes, we can begin to identify components of these pathways through pharmacological and genetic manipulations. This work was supported by the Jeffress Memorial Trust and VMI.

168 Poster : Olfactory Transduction

VOLATILE ANESTHETICS MAY INTERACT DIRECTLY WITH OLFACTORY RECEPTORS IN THE RAT

Peterlin Z.[1], Kini A.[1], Ishizawa Y.[2], Zhang X.[1], Eckenhoff R.[2], Firestein S.[1] [1]*Biological Sciences, Columbia University, New York, NY;* [2]*Department of Anesthesia, University of Pennsylvania Medical Center, Philadelphia, PA*

Many mechanisms of action have been proposed for the complex effects of volatile anesthetics (VAs), including their direct interaction with the ligand binding pocket of G protein coupled receptors (GPCRs). Since VAs have characteristic odors, we reasoned that they may interact with members of the olfactory receptor family of GPCRs. We tested rodent olfactory sensory neurons (OSNs) with a variety of VAs at pharmacologically relevant concentrations. Calcium imaging of acutely dissociated rat OSNs confirmed that the VA halothane activated OSNs in a dose dependant manner, leading to an increase in intracellular calcium which was kinetically similar to odorant induced responses. Consistent with the canonical olfactory transduction pathway, this influx required extracellular calcium and could be modulated by agents affecting the second messenger cascade. Only a subset (9%) of OSNs were activated by halothane, and this population could be further subdivided based on the neuron's response profile to a panel of odorants. This suggests that halothane interacts with a varied, though limited, number of olfactory receptors (ORs). Electrophysiological recordings demonstrated adaptation consistent with receptor mediated action. Further, the halothane response could be reversibly and competitively blocked by receptor antagonists in select OSNs. These results encourage the use of an olfactory based screening system to identify particular ORs as models for the binding of VAs to GPCRs elsewhere in the body.

169 Poster : Olfactory Transduction

THE INTERACTION OF OMP AND BEX REVEALS AN OMP DIMER

Koo J.[1], Margolis F.L.[1] [1]*Anatomy and Neurobiology, University of Maryland at Baltimore, Baltimore, Maryland*

Bex, brain expressed X-linked protein, has been identified as a protein that interacts with olfactory marker protein (OMP). We have confirmed this Interaction between Bex and OMP by in vitro binding assays and by immuno-colocalization experiments. To evaluate further the interaction between OMP and Bex in vivo, co-immunoprecipitation experiments were performed. Surprisingly, when lysates from OMP and Bex transiently co-transfected cells were analyzed using immunoprecipitation, two forms of OMP (19-kDa and 38-kDa) were observed with anti-OMP antibody. The 38-kDa form was selectively co-precipitated in the immuno-complex with Bex. To investigate the 38-kDa OMP, we purified it using affinity chromatography and then characterized it by in-gel tryptic digestion, mass spectrometry, and two-dimensional electrophoresis. Taken together, these data suggest the 38-kDa protein might be an OMP homodimer that was produced through post-translational modification. To verify that the OMP dimer is present in vivo, olfactory tissues from wild-type mice (OMP+/+) and OMP null-mice (OMP-/-) were evaluated by Western analysis. We observed that the OMP dimer occurs at a low level in wild type olfactory epithelium, at about 1% of the OMP monomer. Preliminary data indicate the OMP dimer has a short half-life. Our results identifying an OMP dimer indicate that OMP may function as a dimer in its interactions with Bex in vivo. Supported in part by NIH DC 00347 and DC 03112.

170 Poster : Olfactory Transduction

G-PROTEIN LIKE IMMUNOREACTIVITY ON THE CHELAE AND AESTHETASC HAIRS OF THE LATERAL ANTENNULES IN CRAYFISH (*ORCONECTES RUSTICUS*).

Belanger R.M.[1], Moore P.A.[1], Zielinski B.[2] [1]*Laboratory for Sensory Ecology and the J.P. Scott Center for Neuroscience, Mind and Behavior, Bowling Green State University, Bowling Green, OH;* [2]*Department of Biological Sciences, University of Windsor, Windsor, Ontario, Canada*

Heterotrimeric G-proteins mediate signaling processes and are highly conserved from mammals to invertebrates. Knowledge of the G-protein distribution in crayfish, *Orconectes rusticus*, is useful for examining the location of potential receptor sites on the lateral antennules and chelae. This information can be used to help determine the role of olfaction in certain behaviors. In this study, we used antibodies against G-proteins, $G_{\alpha s/olf}$ and $G_{\alpha 0}$, to identify the location of each protein type on the aesthetasc hairs of the lateral antennules of crayfish. We also stained chelae of the first walking leg and examined the biting edge for G-protein localization. Scanning electron microscopy studies demonstrated that sexually mature males have an increased number of both feathered receptors and filiform hairs on their chelae when compared to immature males and females. $G_{\alpha s/olf}$ like immunoreactivity was found on the feather receptors of both the chelae and lateral antennules. $G_{\alpha s/olf}$ and $G_{\alpha 0}$ like immunoreactivity was located on the aesthetasc hairs of the proximal, medial, and distal annulae. Further analysis will be performed to examine the distribution of each protein type in these regions. A western blot analysis will be performed to detect the presence of the G-proteins in the asesthetasc hairs and feathered receptors of the lateral antennules and feathered receptors of the chelae.

171 Poster : Olfactory Transduction

MOLECULAR CLONING OF A NEW GABA RECEPTOR SUBUNIT FROM THE LOBSTER OLFACTORY ORGAN

Hollins B.[1], McClintock T.S.[1] [1]*Dept. of Physiology, University of Kentucky, Lexington, KY*

In the lobster, presynaptic inhibition of odor signals is mediated by ionotropic GABA and histamine receptors (Wachoviak et al , Microsc. Res. Tech. 58:365-375, 2002). We previously isolated a cDNA clone encoding a GABA receptor subunit (Lobster GABA1) that is likely a component of one of these receptors. Here, we report the isolation of a cDNA encoding a second GABA receptor subunit that is also expressed in the lobster olfactory organ. Lobster olfactory organ cDNA was used to generate a plasmid library containing 300 bp inserts that were generated by PCR using degenerate primers. Selected inserts were sequenced and tested for homology using blast analysis at the NCBI web site. 5 prime and 3 prime RACE reactions were performed on one positive insert to generate the full-length clone. Expression of the clone was tested by PCR on cDNA from several lobster tissues. In situ hybridization was performed on lobster olfactory organ using 50 micron vibratome slices and dig RNA probes. The full-length clone consists of 2769 bp that encodes a 481 amino acid protein. The protein shows 34% identity to lobster GABA1, 54% identity to mouse beta1 subunit, and 67% identity to drosophila LCCH. In situ hybridization verified expression of the subunit in olfactory receptor neurons. Qualitative PCR indicated that the subunit is also expressed in other lobster tissues. The presence of both lobster GABA1 and lobster GABA2 in olfactory receptor neurons suggest that they are components of the same receptor and that invertebrate GABA receptors like their mammalian counterparts are hetero-oligomeric. Supported by NIH grant 5R03Dc04433-02

172 Poster : Olfactory Transduction

MODELING DIFFUSION OF SECOND MESSENGERS IN OLFACTORY CILIA

Flannery R.[1], French D.[1], Kleene S.J.[1] [1]*University of Cincinnati, Cincinnati, Ohio*

Two ion channel types, a cyclic-nucleotide-gated channel and a calcium-activated chloride channel, are sequentially activated during an odorant response. The majority of these ion channels are localized to the cilia of olfactory neurons. The locations and specific channel densities of each channel type within the cilia, which are unknown, are expected to be important for the efficiency and kinetics of signal transduction. We are modeling diffusion of second messengers in an olfactory cilium along with patch-clamp experiments to determine the spatial distribution of ion channels in the ciliary membrane. In our experimental setup, second messengers diffuse down the length of an isolated cilium starting from the open end, activating channels along the length. We have monitored the time course of activation of these channels using patch-clamp experiments in isolated cilia. The location of the channels is determined by matching the results with a computational model of diffusion in the cilium. This work was supported by NIH grants R01 DC00926 and F31 DC006121

173 Poster : Olfactory Transduction

EFFECTS OF CYCLIC AMP ON CULTURED OLFACTORY RECEPTOR NEURONS AND ON OLFACTORY SENSILLA OF THE HAWKMOTH *MANDUCA SEXTA*

Stengl M.[1], Flecke C.[1], Dolzer J.[1] [1]*Animal Physiol., University of Marburg, Marburg, Germany*

Circadian rhythms of biogenic amines appear to modulate insect olfactory sensilla (OS) via cAMP-dependent mechanisms (Roeder 1999, Prog. Neurobiol. 59:533). To examine cyclic AMP-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). Perforated patch clamp recordings of cultured ORNs distinguished 11 types of currents by their current-voltage relations. Application of 8-bromo-cAMP (8bcAMP) decreased the cation currents ILL and Icat?. Icat? shares properties with a protein kinase C-dependent non-specific cation current, and ILL is a long-lasting cation current, which activates with depolarization and inactivates slowly with hyperpolarization. We hypothesize that ILL and Icat? are activated via long-lasting rises of intracellular Ca2+ during adapting pheromone stimulation. Preliminary results of extracellular tip recordings of bombykal (BAL) stimulated pheromone-sensitive OS also suggests an influence of cAMP on pheromone transduction. During perfusion of the sensillar lumen of BAL-stimulated OS (10 µg BAL) with 10 mM of 8bcAMP the sensillar potential increased continuously over a period of 3h while the action potential response remained unaltered at a high frequency. In contrast to 8bcGMP which targets the spike generator, 8bcAMP appears to act on the outer dentritic segment. Current extracellular recordings challenge the hypothesis whether cAMP and octopamin disadapt or sensitize ORNs. Supported by DFG grants STE 531/10-1,2,3.

174 Poster : Olfactory Transduction

CYCLIC AMP-INDEPENDENT AND CYCLIC AMP-DEPENDENT OLFACTORY TRANSDUCTION IN *XENOPUS LAEVIS* TADPOLES

Manzini I.[1], Schild D.[1] [1]*Department of Molecular Neurophysiology, University of Goettingen, Goettingen, "N/A", Germany*

Whether odorants are transduced by only one or more than one second messenger has been a long-standing question in olfactory research. Here we give an answer to this question using a novel preparation, the *Xenopus laevis* tadpole mucosa slice. We show that some olfactory receptor neurons (ORNs) respond to stimulation with amino acids with an increase of the intracellular calcium concentration $[Ca^{2+}]_i$. In order to see whether or not these responses were mediated by the cAMP transduction pathway we applied forskolin or the membrane-permeant cAMP analogue pCPT-cAMP to the olfactory epithelium. The ensemble of ORNs that was activated by amino acids markedly differed from the ensemble of neurons activated by forskolin or pCPT-cAMP. Less than 6% of the responding ORNs showed a response to both amino acids and the pharmacological agents activating the cAMP transduction pathway. We conclude that ORNs of *Xenopus laevis* tadpoles have both cAMP-independent and cAMP-dependent olfactory transduction pathways.

175 Poster : Olfactory Transduction

ROLES OF CYCLIC NUCLEOTIDE AND INOSITOL 1,4,5-TRIPHOSPHATE PATHWAYS MEDIATING RESPONSIVENESS TO FOOD AND PHEROMONAL ODORS IN GOLDFISH OLFACTORY RECEPTOR NEURONS

Sato K.[1], Sorensen P.W.[1] [1]*Fisheries, Wildlife and Conservation Biology, University of Minnesota, St. Paul, Minnesota*

Responsiveness of vertebrate olfactory receptor neurons (ORNs) to odorants is thought to be mediated by multiple second-messenger pathways which involve activation of either adenylyl cyclase (AC)/cAMP, or phospholipase C (PLC)/inositol 1,4,5-triphosphate (IP_3). Last year, we reported that goldfish ORNs are specifically tuned to single classes of odorants including a food odor (amino acids), and two types of sex pheromone (sex steroids and F prostaglandins). To determine whether the high chemospecificity of these ORNs is mediated by different transduction pathways, we have recorded EOG responses and extracellular single-unit activity to these 3 odorants and the effect of pharmacological agents including antagonists of AC and PLC on this activity. Although application of U-73122, an antagonist of PLC, suppressed EOG responses to all 3 odorants in a reversible manner, it exerted a significantly greater effect on amino acid responses. In contrast, application of an AC antagonist (SQ22536) had no effect on EOG responses to any odorant. Further, application of cell membrane permeable cAMP did not stimulate an EOG, whereas 8-br-cGMP produced a significant response. Although forskolin, an AC activator, was a potent stimulus, neither U-73122 nor SQ22536 depressed EOG responses to it. Together, these results suggest that IP_3 pathways mediate responses of goldfish ORNs to both food and pheromonal odorants, and that a cGMP pathway activated by forskolin may also be involved. We are now investigating the latter possibility. NIH/DC03792

176 Poster : Olfactory Transduction

FUNCTIONAL AND STABLE RECONSTITUTION OF AN OLFACTORY RECEPTOR-ACTIVATED cAMP SIGNALLING PATHWAY IN HUMAN HELA CELLS

Chirokova E.[1], Schmiedeberg K.[1], Bedner P.[2], Niessen H.[2], Willecke K.[2], Krautwurst D.[1] [1]*Molecular Genetics, German Institute of Human Nutrition, Bergholz-Rehbrücke, Germany;* [2]*Genetics, University Bonn, Bonn, Germany*

The majority of olfactory receptors (OR) trigger odorant-induced calcium signals *via* a cAMP/cyclic nucleotide-gated (CNG) channel signalling pathway in olfactory sensory neurons. To reconstitute OR signal transduction in a heterologous system, we established a cell line, stably expressing the olfactory CNGA2 channel. By conducting calcium, CNGA2 serves as a reporter for a variety of stimuli that increase the intracellular concentration of cyclic nucleotides. In HeLa-Cx43/CNGA2 cells, the β-adrenergic agonist isoproterenol triggered calcium influx-based fluorescence signals. The odorant (-)citronellal and the C-type natriuretic peptide elicited a calcium influx into HeLa-Cx43/CNGA2 cells, stably expressing the OR MOR118-1, or when transfected with DNA encoding the particulate guanylyl cyclase-B, respectively. Pretreating cells with forskolin, thapsigargin, or overexpression of G protein αs increased the signalling efficacy of both, MOR118-1 and β-adrenergic receptors, while expression of Gαolf was more beneficial for MOR118-1 signalling. In summary, we established a cell line to monitor changes in intracellular cAMP/cGMP levels, allowing the determination of EC_{50}-based odorant profiles for OR. Our data support a central role of Gαolf in OR signalling, and suggest that employing olfactory signal transduction components will facilitate the functional identification and characterization of OR in heterologous cell systems, thus increasing an understanding of odorant coding.

177 Poster : Olfactory Transduction

MODULATION OF THE NATIVE AND RECOMBINANT OLFACTORY CYCLIC NUCLEOTIDE-GATED CHANNEL BY MEMBRANE PHOSPHOINOSITIDES

Zhainazarov A.[1], Spehr M.[2], Wetzel C.[2], Hatt H.[2], Ache B.W.[1] [1]*Whitney Laboratory, Center for Smell and Taste, McKnight Brain Institute, University of Florida, Gainesville, FL;* [2]*Ruhr-Universitaet Bochum, FRG, Bochum, Germany*

Phosphoinositide signaling regulates the level of odorant-activated calcium in rat ORNs, and appears to act by modulating the cyclic nucleotide signaling pathway (Spehr et al., 2002). The mechanism(s) through which phosphoinositides modulate the cyclic nucleotide pathway is unknown, although since phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P3] modulates the action of forskolin (ibid.), modulation presumably occurs downstream of the receptor. Here we show that PI(3,4,5)P3 or phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] applied to the intracellular side of cell-free membrane patches from the dendritic knob of acutely dissociated rat ORNs inhibits activation of the native CNG channel by downregulating the sensitivity of the channel to cAMP. We also show this same effect on the recombinant olfactory CNG channel heterologously expressed in HEK293 cells. These findings suggest that phosphoinositide signaling can modulate excitation of rat ORNs by directly regulating the sensitivity of the olfactory CNG channel to the excitatory second messenger, cAMP. Supported by grants from the NIDCD (BWA), the DFG (HH), and the AvH Foundation.

178 Poster : Olfactory Transduction

CATION-DEPENDENT RECTIFICATION IN THE LOBSTER OLFACTORY SODIUM-GATED CATION CHANNEL

Bobkov Y.V.[1], Ache B.W.[1] [1]*The Whitney Laboratory, Center for Smell and Taste, and McKnight Brain Institute, University of Florida, Gainesville, FL*

Many non-selective cation channels involved in sensory transduction, including the lobster olfactory sodium-gated channel (SGC), cyclic nucleotide-gated channels and some transient receptor potential channels, show double rectification in their current-voltage relationships in the presence of divalent cations. We took advantage of the large single channel conductance of the lobster olfactory SGC to elucidate the potential mechanism of this phenomenon. Single-channel recordings reveal that the channel briefly occupies several subconductance states from the fully open state. $[Ca^{2+}]_i$ or $[Mg^{2+}]_i$ does not alter open state probability or unitary current amplitude, but in $[Ca^{2+}]_i$>3μM or $[Mg^{2+}]_i$>50μM, depolarization increases the probability of fast transitions between substates, although the short lifetime of the substates makes it difficult to quantify the kinetic parameters of the effect. The increased frequency of fast transitions between channel substates at more depolarizing voltages would be expected to decrease the mean integral current in a voltage-dependent manner and account for the observed rectification. We are exploring the potential implication of this phenomenon for the detailed role of the lobster olfactory SGC, and possibly other non-selective cation channels, in olfactory transduction. Supported by a grant from the NIDCD.

179 Poster : Olfactory Transduction

COMPARISON OF IH-CHANNELS FROM INVERTEBRATE OLFACTORY RECEPTOR NEURONS

Gisselmann G.[1], Bobkov Y.V.[2], Marx T.[1], Wetzel C.[1], Neuhaus E.M.[1], Gamerschlag B.[1], Ache B.W.[2], Hatt H.[1] *[1]Ruhr-Universität Bochum, Bochum, Germany; [2]Whitney Laboratory and McKnight Brain Institute, University of Florida, Gainesville, Florida*

Cyclic nucleotides play an essential role in visual and olfactory transduction in many species. We therefore used RT-PCR to discern the presence of ion channels that are gated or modulated by cyclic nucleotides in lobster, honeybee and fruit fly olfactory receptor neurons (ORNs). ORNs of all three species express homologs of members of the Ih (HCN, HAC) family of ion channels that are activated by both hyperpolarization and cyclic nucleotides. When recombinantly expressed in HEK293 cells, cloned AMIH (*A. mellifera*), DMIH (*D. melanogaster*) and PAIH (*P. argus*) gave slowly activating, non-inactivating inward currents under whole-cell voltage-clamp in response to hyperpolarizing voltage steps. cAMP shifted the activation curve to more positive values within the normal resting potential range. The general properties of all three I_h channels were similar, but the channels differed in critical details. In particular, cAMP shifted the activation curve of PAIH significantly more than AMIH. Native I_h-currents in lobster and honeybee ORNs mimicked the recombinant ones. Western blotting localized the expression of PAIH to the olfactory sensilla. The physiological properties of these channels, and the presence of at least PAIH in the transduction zone, suggest a potential a role for these channels in olfactory transduction. Supported by grants from the DFG (HH and GG), the NIDCD (BWA), and the AvH Foundation.

180 Poster : Olfactory Transduction

TARGETING VECTORS FOR HCN1 AND HCN4 KNOCK OUT MICE AND INVESTIGATION OF DISTRIBUTION PATTERNS OF HCN ISOFORMS IN RODENT NASAL EPITHELIUM

Pickenhagen A.[1], Gisselmann G.[1], Wetzel C.[1], Hatt H.[1] *[1]Lehrstuhl fuer Zellphysiologie, Ruhr-Universitaet, Bochum, Germany*

I_h currents are generated by hyperpolarization activated cyclic nucleotide gated (HCN) channels. These proteins are coded by a single gene in invertebrates, and by four (HCN1-4) in vertebrates. Activated by both hyperpolarization and cyclic nucleotides, they show weak selectivity for K^+ over Na^+. I_h is crucial in setting the resting membrane potential and in the control of cell excitability, and is present ubiquitously in many loci of the heart and brain. With relevance to chemical senses, I_h channel mRNA has been detected in the antenna of various arthropods, in the olfactory bulb (HCN1,2,4), as well as in taste cells of rat vallate papilla (HCN1,4). Also, native I_h currents were characterized in the ORNs of rat, lobster and honey bee. It has been suggested that the effects of I_h on odor sensitivity is modulated by dopamine. In order to investigate the relevance of I_h channels for odorant transduction in vertebrates, we plan to make knock out mice for HCN1 and HCN4. For the purposes of a conditional gene targeting event, we are constructing a targeting vector such that exon 2 (HCN1) and exon 4 (HCN4) are flanked by loxP sites. This mouse line will then, after homologous recombination, be crossed with a lineage expressing the recombinase (Cre), resulting in the deletion of the target gene segment only in offspring cells expressing Cre. Further, to identify the different distribution of the four mammalian HCN isoforms in the olfactory system, slices from rodent nasal epithelium as well as single ORNs were investigated.

181 Poster : Olfactory Transduction

RECORDINGS OF Ca^{2+}-DEPENDENT K^+ CHANNELS ACTIVATED BY ODORS IN 'ON CELL' AND EXCISED TOAD CILIARY PATCHES; COMPLEMENTARY LIPID BILAYER STUDIES

Delgado R.[1], Castillo K.[2], Wolff D.[3], Bacigalupo J.[3] *[1]Millennium Institute for Advanced Studies CBB, Santiago, B/A, Chile; [2]Faculty of Sciences, Univ of Chile, Santiago, Chile; [3]Millennium Institute for Advanced Studies CBB, Santiago, Chile*

Excitatory odor responses involve a cyclic nucleotide gated channel (CNGC) and a Ca2+-dependent Cl- channel, both of which are confined to the chemosensory cilia. Previous work from our laboratory provided evidence that a Ca2+-dependent K+ (KCa) conductance is responsible for odor inhibition in toad (C. caudiverbera) olfactory receptor neurons (ORNs), generating a hyperpolarizing receptor potential. We found a similar K+ conductance in the rat. (Morales et al, Proc. R. Soc. London B. 257:235, 1994; Sanhueza et al, Am. J. Physiol. Cell Physiol.:279:C31, 2000). Recently we demonstrated the presence of Ca2+-dependent K+ channels in toad chemosensory cilia, by recording from excised ciliary patches and by immunochemical evidence. Here we show that KCa channels present in the chemosensory cilia membrane can be activated by focal ciliary stimulation with odorants (isovaleric acid, cadaverine and pyrazine). After patch excision, the same K+ channel was activated by μM Ca2+. This channel has 30 and 60 pS subconductance states. A similar channel was recorded in lipid bilayers doped with purified chemosensory ciliary membranes, in μM Ca2+ and symmetrical K+ solutions. These results strongly support the participation of Ca2+-dependent K+ channels in chemosensory transduction of olfactory receptor neurons. Funded by MIDEPLAN ICM P99-31-F, FONDECYT 1020964.

182 Poster : Olfactory Transduction

MITOCHONDRIAL INFLUENCE ON CALCIUM AND ACTION POTENTIAL FIRING RATE IN OLFACTORY RECEPTOR NEURONS OF *RANA PIPIENS*

Buntinas L.[1], Koutalos Y.[2], Restrepo D.[1] *[1]Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO; [2]Physiology and Biophysics, University of Colorado Health Sciences Center, Denver, Colorado*

Because of the spatiotemporal uptake and subsequent release of calcium by mitochondria, they have been hypothesized to act as memory storage devices. The role of mitochondria in signal transduction in olfactory receptor neurons (ORNs) is unknown and is the focus of this study. We attempted to determine if mitochondria are involved in transient calcium regulation and in modulating action potential firing rate in ORNs. We hypothesize that mitochondria buffer Ca^{2+} functioning to modulate action potential output of the neuron. Intracellular calcium imaging and loose patch electrophysiology were used to measure intracellular calcium and action potential generation of isolated frog ORNs in the presence and absence of mitochondrial inhibition. ORNs had a 50% higher intracellular calcium concentration in the presence of antimycin A and oligomycin when stimulated with high K^+. This seems to indicate that mitochondrial calcium buffering is significant for cytosolic calcium regulation. Also, mitochondrial inhibition decreased the firing rate of action potentials in ORNs. This suggests that mitochondria might affect Ca^{2+} in the vicinity of Ca^{2+}-activated K^+ channels to decrease action potential output. This work was supported by: Advanced Training in Basic Neuroscience 5 T32 NSO7083 (institutional training grant).

183 Poster : Olfactory Transduction

CHARACTERIZATION OF THE PLASMA MEMBRANE Ca^2-ATPases IN MOUSE OLFACTORY AND VNO EPITHELIA

Cusick M.[1], Delay R.[2], Van Houten J.[3] [1]*University of Vermont, Burlington,VTt;* [2]*Biology Department, University of Vermont, Burlington, VT;* [3]*Zoology/Cell and Microbiology, University of Vermont*

Control of Ca_i is critical in olfactory and VNO receptor neurons because of its roles in signal transduction. Ca_i is controlled by binding proteins, intracellular pumps, and extrusion from the cell by the Na^+/Ca^{2+} transporter and plasma membrane Ca pumps (PMCAs). Imaging of isolated ORNs shows that calcium levels can return to basal levels even in the absence of transporter function (Delay & Dionne, unpublished), pointing to a role for PMCAs in Ca removal. Mammals have 4 PMCA isoforms, each with different kinetic properties for serving different physiological tasks. Western blots of mouse tissues show expression of isoforms 1-4 in olfactory tissue and isoforms 1,2,4 in VNO, with differences in quantity and splice variants. Immunocytochemistry with the pan-pump antibodies show distinct staining patterns in the olfactory and VNO epithelia: intense in the apical region of the olfactory epithelium but not in the respiratory epithelium; none apparent in the cilia; faint in the region of the neuron cell bodies. Use of specific antibodies shows: in VNO intense staining for isoforms 1 and 2, and little or none for isoforms 3 and 4; in olfactory epithelium intense staining for isoform 1, less for isoform 2, and weak for isoforms 3 and 4; in respiratory epithelium, weak to no staining of any isoform. Isoform 1 is ubiquitous, but the intense staining in VNO and olfactory epithelia for PMCA isoform 2 (highest in affinity for Ca/CaM of the PMCAs) implies that this isoform performs specialized function, perhaps in the dendritic region of the sensory neurons. Supported: NIH DC00721, P20RR16435, NCI PHS22435.

184 Poster : Olfactory Transduction

SPATIAL RELATIONS BETWEEN OLFACTORY SENSORY NEURONS REVEALED IN GENE-TARGETED MICE

Grosmaitre X.[1], Ma M.[2], Vassalli A.[3], Mombaerts P.[3], Shepherd G.M.[1] [1]*Neurobiology, Yale University, New Haven, CT;* [2]*Neuroscience, University of Pennsylvania, Philadelphia, PA;* [3]*Rockefeller University, New York, NY*

Spatial distribution of individual olfactory sensory neurons (OSNs) responding to a given odorant has not been characterized systematically despite its importance for understanding peripheral coding mechanisms in olfaction. We wished to test if OSNs with similar response spectra tend to cluster, reflecting developmental mechanisms. We have performed functional studies in gene-targeted mice in which all the OSNs expressing the MOR23 receptor were labeled with tauGFP. Using patch clamp recording of the dendritic knobs of individual OSNs in the intact olfactory epithelium, we found that all GFP-labeled cells responded to lyral, a known ligand of the MOR23 receptor. The Hill coefficient of the dose-response curve was significantly lower than that reported for isolated OSNs, suggesting that OSNs in the intact epithelium detect a broader range of odor concentrations. Lyral-induced responses showed kinetics similar to those induced by IBMX in both green and non-green cells, indicating an unaltered cAMP signaling cascade in these GFP-labeled cells. This result was confirmed by inhibition of the response during application of an adenylyl cyclase inhibitor . One fourth of the GFP-labeled cells also responded to other odorants. Using calcium imaging techniques allowing monitoring of the activities of many cells with spatial relations intact, we observed that GFP-labeled cells were surrounded by other cells also responding to lyral. Supported by: NIDCD, Whitehall Foundation (MM); NIH (PM); and NIDCD, Human Brain Project, MURI (GMS).

185 Poster : Olfactory Transduction

OPTICAL IMAGING OF INTRACELLULAR Cl^- IN MOUSE OLFACTORY NEURONS, DYNAMIC CHANGES WITH ODOR STIMULATION.

Delay R.[1], Verret T.J.[2] [1]*Biology Department, University of Vermont, Burlington, VT*

In mammals, the majority of excitatory odor transduction is mediated by ligand activation of an odor receptor which leads to an increase in cAMP, followed by the subsequent activation of a cAMP gated channel. Calcium influx through this channel opens a Ca^- dependent Cl^- channel. If intracellular Cl^-, [Cl]i, is high the resulting Cl^- current can greatly enhance the response to an odor. If [Cl]i is low then activation of a Ca^- activated Cl^- current will result in an influx of Cl^- that will hyperpolarize the cell. Thus [Cl]i is critical in determining whether the odor response is excitatory or inhibitory. To study the relationship of [Cl]i and odor responses, a series of experiments were undertaken using a Cl^- sensitive fluorescent dye. Isolated olfactory cells were loaded with membrane permeant form of 6-methoxy-N-ethylquionolinium iodide (MEQ). MEQ fluorescence is inversely proportional to Cl^- concentration. MEQ is not a ratiometric dye. To determine [Cl]i required calibrations for each cell using a set of Cl^- bath standards and Cl^- ionophores to determine [Cl]i. Our results indicated a wide range of [Cl]i exist in isolated OSNs (range: 20 mM to 145 mM; mean: 91 mM). Openlab software (Improvision) was used to detect the changes in the level of fluorescence when various stimuli were applied. Stimulation with either IBMX/forskolin, to increase intracellular cAMP levels, or odor mixtures elicited changes in MEQ fluorescence that appeared to correlate with initial [Cl]i, increased if low and decreased if high. Thus, [Cl]i appears to have an integral role in odor transduction. Supported by NIH grant P20RR16435.

186 Poster : Olfactory Transduction

P2Y RECEPTORS IN AN OLFACTORY CELL LINE (ODORA)

Washburn K.[1], Liu G.[1], Turner T.[1], Talamo B.R.[1] [1]*Neuroscience, Tufts University, Boston, MA*

Activation of purinergic receptor (P2R) subtypes is reported to modulate odor sensitivity in mouse olfactory epithelial slices (C.C. Hegg and M.T. Lucero, Chem Senses 27:A107, 2002). ATP also activates cytosolic Ca^{2+} increases in *Odora*, a rat olfactory-derived cell line, as we have shown previously (Liu et al, Chem Senses, 26:1126, 2001. ATP and UTP activate phospholipase C, generate inositol (1,4,5) trisphosphate (IP3), and mobilize Ca^{2+} through IP3 receptors (blockade by xestospongin C). In the present studies, Ca^{2+} imaging experiments with fura-2 were used to characterize the P2R subtypes present in differentiated Odora cells and to examine regulation of the response. Differentiated *Odora* cells exhibit two distinct pharmacological profiles in response to nucleotides. 95% of *Odora* cells respond to UTP and ATP, which act with equal potency (Ka = 3.9x10-6 M and 3.2x10-6 M, respectively), consistent with the properties of the rat P2y2-receptor. UTP was 30% more efficacious than ATP. A subset of cells also shows responses indicative of the P2y1-receptor (potency: 2-methylthioATP (2-MeSATP) = 2-methylthioADP > ADP > ATP). 47% of *Odora* cells respond to 2-MeSATP (maximal at 1.0 μM) and 28% respond to ADP (maximal at 1.0 μM). *Odora* cells do not respond to UDP.We show also that preincubation with 1 μM phorbol dibutyrate reduces the response to 3 μM UTP. This appears to be primarily through activation of protein kinase C (PKC), since the effect of phorbol ester can be blocked partially by the selective PKC inhibitor BIM-1. Thus, signaling through PKC could modulate the effect of ATP on odorant response.

187 Poster : Olfactory Transduction

GONADOTROPIN-RELEASING HORMONE MODULATES THE VOLTAGE-ACTIVATED SODIUM CURRENT IN NECTURUS OLFACTORY NEURONS

Zhang W.[1], Delay R.[1] *[1]Biology Department, University of Vermont, Burlington, VT*

The terminal nerve extends from basal forebrain to the nasal cavity and has been shown to contain gonadotropin-releasing hormone (GnRH). The function of the terminal nerve is unknown but it has been suggested that it modulates the function of olfactory neurons. We used the perforated configuration of the patch clamp technique to examine the effects of GnRH on isolated olfactory sensory neurons (OSNs). GnRH had no direct effect on the membrane current but modulated the voltage-activated TTX-sensitive sodium current (INa). Approximate 60% of the OSNs responded to GnRH within 1 min of application by decreasing the magnitude of INa. Initial responses to GnRH were inhibitory although in one group of cells they were potentiated with continual application (~ 10 min). Due to the time course this effect had to be mediated by a second messenger pathway. Possible second messenger pathways are PKA, PLC, tyrosine kinase, phosphoinositide 3-OH kinase (PI3K). One activator, a cAMP analog, mimicked the effect of GnRH. Inhibitors of PKA, PKC, PLC, PI3K and tyrosine kinase were all able to inhibit INa but none of them blocked the GnRH effect completely. INa in OSNs appears to be modulated by multiple second messenger pathways. There are multiple phosphorylation sites on the sodium channel, depending on the isoforms expressed, which can be phosphorylated by different protein kinases (Catteral, 2001). The modulation of voltage-activated sodium current by GnRH may involve multiple pathway activation. Experiments are currently underway to examine this possibility. Supported by a grant from NIH R03DC03912 and P20RR16435

188 Poster : Olfactory Coding in the Periphery

EVOLUTION OF THE OLFACTORY CODE IN THE *DROSOPHILA MELANOGASTER* SUBGROUP

Stensmyr M.[1], Hansson B.[1] *[1]Swedish University of Agricultural Sciences, Alnarp, Sweden*

We have studied the evolution of the olfactory code across all the nine species of the *Drosophila melanogaster* subgroup, an excellent target for such studies. The fruitfly, *D. melanogaster*, is a long-standing important model organism in olfactory research. *D. melanogaster* and its eight close relatives have furthermore been the focus of numerous studies regarding speciation and evolution, and thus their phylogenetic relationship and their evolutionary history are fairly well understood. In addition the subgroup contains species with a wide difference in both distribution, endemics to cosmopolitans, as well as in lifestyles, narrow specialists to broad generalists, features that may have a bearing on the olfactory system. We have used electrophysiological recordings from single receptor neurons, so called single-cell technique to determine the response characteristics of a selected set of olfactory receptor neurons. Here we show that the olfactory code, as relayed by the defined subset of receptor neurons is to a large degree conserved among the nine species of the *melanogaster* subgroup. Notable and remarkable changes have however occurred in the *simulans* cluster. These changes, especially pronounced in the two island endemics are likely adaptations for their insular lifestyles and their shifted food preferences. Furthermore we also show that the architecture of the olfactory system is highly conserved.

189 Poster : Olfactory Coding in the Periphery

NEURAL TRACING OF OLFACTORY SENSORY NEURONS IN LAND SNAIL (EOBANIA VERMICULATA)

Mazzatenta A.[1], Pelosi P.[2], Cellerino A.[3] *[1]Biophysic, International School for Advanced Studies, Trieste, Italy; [2]Biotechnology and Ag. Chemistry, Pisa University, Pisa, Italy; [3]Neurophysiology Institute, Scuola Normale Superiore, Pisa, Italy*

The sense of smell informs an organism about the chemical composition of the external environment. The precise connections between neurons are essential for translating neuronal activity into meaningful neural codes. Until now, the olfactory system neural network has been investigated mainly in mammals and insects, where olfactory sensory neurons project with precision to a given glomerulus. A topographical map representing these connections is organized within the olfactory bulb or antennal lobe. We investigated the organization of the olfactory system in the land snail, *Eobania vermiculata*. Olfactory sensory neurons and glomeruli cells were traced using DiI and DiO. We were able therefore to visualize neuronal pathways from axonal to dendritic projection. It seems from the data, that there is a clear segregation of the olfactory sensory neurons in the olfactory sensory epithelium. We also labeled glomeruli cells, counted the numbers of individual glomeruli and highlighted the olfactory nerve. Finally, a three-dimensional confocal microscopy reconstruction of the olfactory sensory epithelium and glomerulus are presented. These findings suggest that the olfactory region organization in land snail is analogous to that of mammals, but is simplified, suggesting the use of the land snail as a new model to investigate the olfactory system.

190 Poster : Olfactory Coding in the Periphery

CHEMO- AND MECHANOSENSORY RESPONSES BY CRAYFISH (*ORCONECTES RUSTICUS*) ANTENNAE

Urban L.[1], Voigt R.[2], Moore P.A.[1] *[1]Biological Sciences, Bowling Green State University, Bowling Green, OH; [2]Biological Sciences, Bowling Green State University, Bowling Green, Ohio*

Crayfish have been shown to use both chemo- and mechanosensory information to locate food sources. Antennular chemoreceptor cells of crayfish distinguish between different chemical compounds, as well as, there concentrations. After an initial phasic response to an increase in stimulus concentration, chemoreceptor cells quickly adapt and spike frequency reverts to a low tonic level. We investigated the response of chemoreceptor cells of crayfish to 24 diffferent odors. These odors were mixed with a dopamine tracer that allowed us to measure the stimulus concentration profile with high spatial and temporal resolution (IVEC). Cells responded during stimulus onset and once the concentration stopped rising, cells adapted to the constant background within a couple of seconds. In this study, we found that single cells could discriminate between a wide range of different odors.

191 Poster : Olfactory Coding in the Periphery

SENSORY NEURONS IN THE GOLDFISH OLFACTORY EPITHELIUM RESPOND DURING APPLICATION OF A GREAT VARIETY OF STIMULI

Zippel H.P., Albrecht, B, Dolle, H., Foitzik, M. Hamadeh, A. Möller-de Beer, Köhnke, R [1]*Physiology, Georg-August University, Goettingen, Germany, Germany*

Recordings from sensory neurons were made with Platinum-black electrodes. Olfactory stimuli were applied for 15s in 10-7-10-9M concentrations. The effects of a great variety of stimuli, non-familiar and familiar stimuli: preovulatory and ovulatory pheromones, the alarm pheromone, L-amino acids, stereoisomeres of amino acids were investigated in subsequent experimental series. In contrast to olfactory bulb relay neurons the number of sensors neurons not responding to any of the respective investigated stimuli was comparatively high (50-60%). Approximately 20% of sensors showed inhibitions or excitations. One sensory neuron could be excited by one and inhibited by another stimulus. "Generalists" responded during application of a great number of different stimuli, and "specialists" only during one, or one class of stimuli. Structurally nearly similar stimuli (like a- and ß-ionone, stereoisomeres of amino acids) are discriminated by the same percentage of sensory neurons like structurally very different stimuli and stimuli of different classes of stimuli. In contrast to EOG recordings distribution of effects is similar during application of non-familiar and familiar stimuli, and as well during application of amino acids and pheromones in the regenerating epithelium after olfactory nerve axotomy. The olfactory system is extremely mechano-sensitive: After interruption of the laminary (1ml?s-1) flow of tap 76% of sensory neurons show an inhibition, 15% an excitation, and in 9% of cells the activity persists. Supported DFG Zi 112/7-3

192 Poster : Olfactory Coding in the Periphery

EXCITATORY AND SUPPRESSIVE RESPONSES OF CATFISH OLFACTORY RECEPTOR NEURONS IN HIGHLY PURIFIED WATER

Dolensek J.[1], Miklavc P.[1], Valentincic T.[1] [1]*Department of Biology, University of Ljubljana, Ljubljana, Slovenia*

To determine whether the concentration of ions in water bathing the olfactory organ affects the odorant-induced responses to amino acids, the olfactory organs of black bullhead catfish (*Ameiurus melas*) were tested in artificial pond water (APW) and in highly purified water (HPW) (R>18.2 megaΩcm; Milli-Q185, Millipore). The amplitude of the negative electro-olfactogram (EOG) in response to L-norvaline (nVal) was approximately 10 times greater in HPW which contained at the nasal cavity outlet as little as 30±2µg/l sodium, 45±5 µg/l potassium and 12±2µg/l calcium ions than in the APW. Using extracellular electrodes, we compared the responses to amino acid stimuli of twenty-four spontaneously active and eight olfactory receptor neurons (ORNs) lacking spontaneous activity (silent ORNs). In APW, the ratio of excited:suppressed:not responding ORNs was 4:6:2 (N=12) and in the HPW this ratio was 6:4:2 (N= 12). Three silent ORNs that responded to nVal stimulation were detected in the APW, whereas five silent ORNs that responded to nVal were detected in the HPW. In the APW, four ORNs did not show a decline in action potential (ap) amplitude during excitatory responses, whereas a single ORN exhibited decreasing amplitudes of aps. In the HPW, four ORNs did not show a decline in the amplitude of aps during excitatory responses, whereas two ORNs did. In conclusion, ion concentration in the bathing water did not affect ORNs responses to amino acids. This work is supported by Ministry of Education and Science Grant P0-0509-0487 to Tine Valentincic.

193 Poster : Olfactory Coding in the Periphery

A CROSS-SPECIES COMPARISON OF METABOLIC MARKERS IN OLFACTORY EPITHELIUM

Sitthichai A.A.[1], Lucero M.T.[1], Michel W.C.[1] [1]*Physiology, University of Utah, Salt Lake City, UT*

Working under the hypothesis that olfactory tissues from different species have distinct metabolic signatures, we compared concentrations of metabolic markers in the olfactory epithelium from several phyla. We immunostained for alanine, arginine, aspartate, glutamate, glutamine, glycine, glutathione and taurine on squid, lobster, zebrafish, tadpole, and mouse OE. Tissue was fixed in 2.5% glutaraldehyde, embedded in plastic, cut into consecutive 50-500 nm sections, stained with glutaraldehyde-conjugated antibodies and registered using PCI Geomatica software. Quantification of the relative amounts of metabolites allowed us to distinguish several unique metabolic profiles. In vertebrates, aspartate, glutamate, taurine and GSH levels discriminated mature olfactory receptor neurons (ORNs) from other cell types present in the OE; tadpole and zebrafish had the most similar metabolic profiles. Squid ORN metabolic profiles were variable; ORN subtypes had high levels of arginine, aspartate, and glycine. Individual metabolite levels were uniform across lobster ORNs, but ranged from low (taurine) to high (glutamate). In most species, olfactory ensheathing glia, nerve layer, respiratory/support cell and mouse VNO had significantly different metabolic profiles than ORNs. The heterogeneity in the metabolic profiles observed in vertebrates and squid may reflect the presence of mature ORNs and other cells types (basal, immature & supporting cells) whereas in the lobster, mature ORNs are spatially segregated from immature ORNs (Steullet et al. 2000 J Neurosci. 20:3282-94). This work was funded by NIH NINDS grant # P01 NS07938 to MTL & WCM.

194 Poster : Olfactory Coding in the Periphery

STRUCTURE-FUNCTION CORRELATIONS OF TRANSDUCTION IN OLFACTORY RECEPTOR NEURONS IN CATFISH

Rolen S.H.[1], Hansen A.[2], Anderson K.T.[2], Nikonov A.[1], Finger T.E.[2], Caprio J.T.[1] [1]*Biological Sciences, Louisiana State University, Baton Rouge, LA;* [2]*Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, Colorado*

Our previous studies in the channel catfish suggest that a correlation exists between the type of odorant detected, the morphological type of olfactory receptor neuron (ORN) activated, and the specific transduction cascade utilized. However, it is unclear whether each specific class of odorant acts via only a single transduction cascade in only one kind of ORN. We relied on our previously-established odotopic map in the olfactory bulb (OB) to retrogradely label those ORNs projecting to either the bile salt-activated zone or the amino acid-activated zone of the OB. In addition, we used the EOG to assess the effect of pharmacological agents that specifically perturb the cAMP (forskolin; an adenylate cyclase activator) and IP_3 (U-73122, inhibits agonist induced PLC activation) signaling pathways. Forskolin attenuated the response to bile salts and amino acids by 74% and 70%, respectively. Conversely, U-73122 had no significant effect on the bile salt response while reducing the response to amino acids by 63%. Taken together with the anatomical results, these findings indicate that bile salts stimulate primarily ciliated ORNs which utilize the G_{olf}-cAMP transduction pathway. In contrast, amino acid odorants are detected by both microvillous ORNs using an IP_3 pathway, and by ciliated ORNs using the G_{olf}-cAMP system. Thus while some odorants (e.g. bile salts) rely primarily on a single transduction pathway, other classes of odorants (amino acids) can stimulate different ORNs that utilize different transduction cascades. Supported by NIH DC-03792

195 Poster : Olfactory Coding in the Periphery

PHEROMONE RESPONSES IN OLFACTORY RECEPTOR NEURONS OF SEA LAMPREYS (*PETROMYZON MARINUS*).

Teeter J.[1], Lischka F.[1], Li W.[2] [1]*Monell Chemical Senses Center, Philadelphia, PA;* [2]*Michigan State University, East Lansing, MI*

Pheromones have been implicated in sea lamprey migration and reproduction. Bile acids excreted by larval lampreys are used by migrating adults as a cue for selection of a spawning stream. Sexually mature males, and probably females, release pheromones that attract mature conspecifics of the opposite sex and presumably play roles in mate localization, pair-formation and spawning. The larval bile acids, petromyzonol sulfate (PS) and allocholic acid 3-sulfate (ACA), as well as L-arginine and the sex attractant released by mature males, 3-ketoPS, elicited marked EOG responses in adult sea lampreys. Whole-cell recordings were used to characterize odorant responses in olfactory receptor neurons (ORNs) from larval, parasitic and spawning stage lampreys. Responses to PS, L-arg and 3-ketoPS have been recorded from ORNs from all three life stages, suggesting that their behavioral relevance changes during development. Most ORNs responded to either L-arg or a bile acid, although some responding to both were observed in each life stage. The reversal of odor-induced currents near 0 mV, as well as similar currents in response to IBMX and forskolin, are consistent with a cyclic nucleotide-mediated transduction cascade. Only depolarizing (excitatory) responses to L-arg were observed in ORNs from post-metamorphic lampreys, while predominantly inhibitory responses were recorded in ORNs from larvae, indicating a developmental change in response-coupling. Responses to 3-ketoPS were observed in both mature females and males. Supported by NIH DC-04718 and the Great Lakes Fishery Commission

196 Poster : Olfactory Coding in the Periphery

PHARMACOLOGICAL PROFILE OF MAMMALIAN RECEPTORS FOR OCTANAL

Araneda R.C.[1], Peterlin Z.[1], Firestein S.[1] [1]*Columbia University, New York, NY*

It is generally believed that most odor coding is the result of a combinatorial scheme in which receptors recognize multiple related odors and odors are recognized by multiple receptors. From a pharmacological view, a deeper understanding of this mechanism requires defined molecular receptive fields for a number of receptors – are all receptors broadly tuned, what are the gradations of tuning, etc.? To attain this goal we have taken an approach that starts with the ligands rather than specific receptors, by using the response to a common odorant, octanal, as the basis for defining multiple receptor profiles. Relying on the generally well supported belief that each OSN expresses only one receptor, the response profile of each cell corresponds to the pharmacological profile of one particular receptor. We stimulated isolated cells with a panel of odorants, which included octanal, octanoic acid, octanol and cinnamaldehyde among others (all at 30 uM). Using cluster analysis we distinguished several distinct pharmacological profiles for cells that were all sensitive to octanal. Some receptors had a broad molecular range, while others were activated only by octanal. Using a simple statistical analysis we determined that the lower estimate for the maximal number of receptors for octanal is ~65 or 5% of the receptors. This large number of receptors for octanal suggests that, although the peripheral olfactory system is endowed with high sensitivity, discrimination among different compounds likely requires further central processing. Supported by NIDCD

197 Poster : Olfactory Coding in the Periphery

OLFACTORY RECEPTOR ANTAGONISM BETWEEN ODORANTS

Oka Y.[1], Omura M.[1], Kataoka H.[1], Touhara K.[1] [1]*Department of Integrated Biosciences, University of Tokyo, Chiba, Japan*

In the mammalian olfactory system, the detection of thousands of volatile odorants is mediated by several hundreds of different G protein-coupled olfactory receptors (ORs). The main strategy for odorant discrimination is a combinatorial receptor code scheme in that different odorants are recognized by different sets of ORs. The mOR-EG, encoded by the MOR174-9 gene, is a mouse OR that recognizes eugenol, vanillin, and other structurally similar odorants (Kajiya et al., 2001). In this study, competitive antagonists for mOR-EG, which inhibited eugenol-induced Ca^{2+} response in HEK293 cells, were found by screening approximately 500 odorants. Ca^{2+} imaging and single-cell RT-PCR analysis of isolated olfactory neurons demonstrated that the antagonists inhibited endogenous mOR-EG response. Pharmacological properties of the antagonists were determined both in HEK293 cells and single olfactory neurons that expressed mOR-EG. The antagonism of olfactory neuron activities by odorants was observed in a coronal slice of the olfactory epithelium at single-cell level by in situ Ca^{2+} imaging. These results illustrate a molecular aspect in determination of a receptor code for an odorant mixture, which reminisces observation in 60's such that the perceived intensity of an odorant mixture was never as large as the sum of the intensities of its components in psychological experiments. The current study also provides new insight into strategies to modulate perceived odorant quality of, for example, a malodor.

198 Poster : Olfactory Coding in the Periphery

HIGH THROUGHPUT IMAGING OF OLFACTORY NEURONAL RESPONSES TO SINGLE COMPOUNDS AND MIXTURES

Adamek G.[1], Bryant B.[1], Rawson N.[1] [1]*Monell Chemical Senses Center, Philadelphia, Pennsylvania*

If odorant quality is encoded in specific activation patterns of peripheral olfactory receptor neurons (ORNs), then it is necessary to look at the responses of large numbers of cells to adequately determine those patterns. Odorants of dissimilar perceptual quality should activate different neuronal populations, while odorants of similar perceptual quality may activate overlapping subpopulations. ORN specificity has been examined in several mammalian species yet the relative specificity of human ORNs is unknown. Earlier studies in this laboratory with odorant mixtures suggested that ORNS from humans are more selective than those of rat, but also revealed an age-associated loss of specificity. Studies with single odors are needed to better assess ORN specificity, but are not feasible using standard single cell techniques due to low response frequency. We used an automated microscope, microwell plate manipulator and stimulus delivery system to measure calcium responses in human ORNs. With this high throughput system, we can measure responses to 4 stimuli from up to 3000 cells per day. Cells were tested after biopsy or primary culture. The response frequency for single compounds ranges from 1 to 2%, with some cells responding to more than one pure compound, suggesting less than absolute specificity. Mixtures that activate either the cAMP or IP3 pathways stimulated exclusive populations of neurons, confirming earlier findings. This system will enable us to determine the impact of aging and culture environment on the odorant specificity of human ORNs and better understand odorant quality coding. Funded in part by DC000214.

199 Poster : Olfactory Coding in the Periphery

ELECTROPHYSIOLOGICAL ANALYSIS OF ARRESTIN FUNCTION IN PERIPHERAL OLFACTORY SIGNALING IN INSECTS

Merrill C.E.[1], Zwiebel L.J.[1] [1]*Biological Sciences, Vanderbilt University, Nashville, TN*

The ability to adapt to the environment is crucial to maintaining proper function of any sensory system; at the molecular level, this process is referred to as desensitization and results in a progressively weaker response to persistent or repeated stimuli. A superfamily of proteins called arrestins are known to interact with activated G protein-coupled receptors to inhibit further downstream signaling and quench, or arrest, the response. Arrestins have been shown to mediate desensitization of phototransduction in the visual systems of both vertebrates and invertebrates as well as olfactory responses in vertebrates. As invertebrate odorant receptors are also G protein-coupled, it is therefore hypothesized that arrestin genes are likely to regulate peripheral olfactory signaling in insects as well. Recently, our lab revealed that two arrestin genes exist in both visual and olfactory tissues of the malaria vector mosquito *Anopheles gambiae* and of *Drosophila melanogaster*, a model organism which offers abundant genetic and technological approaches for studying the role of arrestins in an intact olfactory system. In order to determine the functional consequences of this dual sensory expression pattern, an expansive electrophysiological analysis has been undertaken to examine peripheral olfactory signaling in arrestin-mutant *D. melanogaster*. Investigating both amplitude and kinetic elements of electroantennogram responses from arrestin-deficient flies allows for construction of a testable hypothesis for the mechanism of arrestin function in peripheral olfactory processing in insects.

200 Poster : Olfactory Coding in the Periphery

ARRESTIN FUNCTION IN OLFACTORY BEHAVIORAL OUTPUTS IN *DROSOPHILA*

Sherertz T.M.[1], Merrill C.E.[1], Zwiebel L.J.[1] [1]*Biological Sciences, Vanderbilt University, Nashville, TN*

Arrestins are a superfamily of regulatory proteins that bind to activated G-protein coupled receptors (GPCRs) to mediate receptor desensitization and endocytosis via clathrin-coated pits. Due to their role in terminating GPCR mediated-signaling, arrestins are likely to have a broad function in the olfactory and other sensory systems by desensitizing GPCRs such as odorant and taste receptors. As expected of genes involved in mediating multiple sensory modalities, our laboratory has identified arrestin genes expressed in both olfactory and visual tissues of *Drosophila melanogaster* and we are utilizing this model system to investigate the role of arrestins in insect olfaction. Since arrestins have been shown to mediate receptor specificity, we hypothesize that disrupting the receptor-arrestin interaction might affect the functional consequences of odorant signal transduction such as the overall behavioral output of the insect. In order to identify olfactory phenotypes associated with the loss of arrestins, we have employed an established larval behavior assay to study attraction responses in wild-type and arrestin deficient strains of *Drosophila*. Data will be presented describing the role of arrestins in larval olfactory behaviors relating to a wide range of chemical odorants. This study substantiates the importance of arrestin's role in olfactory signal transduction and will set the stage for similar experiments in other insects that act as agricultural pests and as vectors for the transmission of insect-borne diseases such as malaria and West Nile virus.

201 Poster : Neurotransmitters in the Olfactory CNS

GRANULE CELL-MEDIATED LATERAL INHIBITION CAN BE DRIVEN BY METABOTROPIC GLUTAMATE RECEPTORS IN THE MAIN OLFACTORY BULB

Heinbockel T.[1], Ennis M.[1] [1]*Anatomy & Neurobiology, University of Maryland School of Medicine, Baltimore, MD*

Olfactory bulb granule cells (GCs) express high levels of the Group I metabotropic glutamate receptor (mGluR) mGluR5. We explored the role of mGluRs in regulating GC activity in mouse MOB slices using whole-cell patch-clamp electrophysiology. The Group I/II agonist ACPD and the selective Group I agonist DHPG depolarized and increased the firing rate of GCs. In the presence of APV, CNQX, Gabazine, which eliminated spontaneous GC firing, DHPG evoked a 5-10 mV depolarization. In voltage clamp, mGluR agonists induced an inward current in GCs that reversed polarity near the potassium equilibrium potential. These results indicate that activation of mGluR5 increases the excitability of GCs, which in turn, should increase GABA release onto mitral cells. In support of this, voltage clamp recordings showed that DHPG increased the frequency of spontaneous IPSCs in mitral cells in the presence of ionotropic glutamatergic receptor antagonists (APV, CNQX). These findings indicate that activation of mGluR5 directly depolarizes GCs, possibly via closure of potassium channels. This mGluR5-mediated excitation increases GABA release from GCs. Taken together, these results suggest that activation of mGluR5 may participate in feedforward and/or feedback inhibition at dendrodendritic synapses between mitral cells and GCs. Support PHS grants: DC03195, DC02588, DC00347 & NS36940

202 Poster : Neurotransmitters in the Olfactory CNS

DISTRIBUTION OF GLUTAMATE RECEPTOR SUBUNITS IN THE OLFACTORY BULB OF ZEBRAFISH, *DANIO RERIO*

Deyoung C.L.[1], Byrd C.A.[1] [1]*Biological Sciences, Western Michigan University, Kalamazoo, Michigan*

The zebrafish is an ideal model for studies of the olfactory system, and is becoming an increasingly popular model in the scientific community. While numerous studies have begun to characterize the olfactory bulb in this species, there is still little known about the structure and function of the different cell types in the zebrafish bulb. Since both ionotropic and metabotropic glutamate receptors appear to play a vital role in the olfactory system of other vertebrates, we examined the potential role that glutamate receptor subunits play in functioning of the olfactory system of zebrafish. Using immunocytochemistry with antibodies to various glutamate receptor subunits, we studied cellular distribution within the zebrafish olfactory bulb. Ionotropic GluR4-IR appeared to localize to juxtaglomerular cells in the glomerular layer (GL), with little or no label in the olfactory nerve layer (ONL) and internal cellular layer (ICL). The cellular distribution of GluR4-IR was reminiscent of anti-tyrosine hydroxylase labeling. Ionotropic GluR1-IR strongly labeled thick fibers in the glomerular layer. Antibody labeling for metabotropic GluR1 appeared to be specific to a subset of large somata in the GL and ICL, presumably mitral cells, as well as numerous small somata throughout these layers. By continuing to examine the distribution of glutamate receptors in the zebrafish olfactory bulb and comparing this to distributions found in other vertebrates, we hope to begin defining the principle function of different cell types in the bulb of this species. Supported by NIH DC04262 and WMU Monroe-Brown Graduate Research Awards.

203 Poster : Neurotransmitters in the Olfactory CNS

FUNCTIONAL EXPRESSION OF NMDA RECEPTORS IN THE DEVELOPING OLFACTORY SYSTEM OF ZEBRAFISH.

Sakata Y.[1], Michel W.C.[1] [1]*Physiology, University of Utah, Salt Lake City, Utah*

In zebrafish, olfactory sensory neurons (OSNs) invade the forebrain early in the second day of development, chemosensory-mediated behavior does not begin until 5 d post fertilization (PF). This suggests that chemosensory competency requires the coordinated development of peripheral olfactory, central neural and motor systems. Towards understand this process, we are examining the functional development of NMDA sensitivity in mitral cells and local interneurons in the nascent olfactory bulb. Taurine (Tau) immunoreactivity identified OSN projections into the developing bulb. To assess NMDA receptor function, zebrafish larvae were decapitated and the heads were exposed to either 5 mM agmatine (AGB) in artificial cerebral spinal fluid alone or AGB and 1 mM NMDA in ACSF for 10 min. AGB is an activity dependent probe capable of entering neurons through open NMDA receptors. After the experiment, the heads were fixed and embedded in plastic. AGB, glutamate, GABA and Tau antibodies, applied to 500nm plastic sections, were visualized with silver intensification. Tau-positive OSNs, GABA-positive inhibitory interneurons and glutamate-positive, GABA-negative mitral cells were first noted during the second day of development. In AGB exposed preps, a few OSNs were labeled in embryos over 32 h PF; no labeling was seen in the brain. In NMDA stimulated preps, excitatory and inhibitory neurons were labeled in all embryos over 32 h PF. Collectively, these results indicate that neuronal maturation and NMDA receptor expression occurs concurrently with the invasion of OSNs into the forebrain. Supported by NIH Grants DC-01418 and NS-07938.

204 Poster : Neurotransmitters in the Olfactory CNS

NMDA R1 SUBUNIT EXPRESSION IN MOUSE OLFACTORY BULB

Josephson E.M.[1], Jennart A.P.[1] [1]*Anatomy, Physiology and Pharmacology, Auburn University, Auburn, AL*

Expression of the glutamate receptor subunit NMDA R1 (NR1) in the mouse (*M. musculus*) olfactory bulb (OB) has not been described. Swiss Webster mice were perfused with saline followed by mixed aldehydes, in phosphate buffer, pH 7.5. Parasagittal sections of whole brain or transverse sections of OB, 30 μm thick, were incubated in a monoclonal antibody against amino acids 660-811 of NR1 or in normal serum alone. Some sections were microwaved for antigen retrieval prior to incubation. Primary antibody was visualized by the streptavidin-biotin-HRP method using DAB as chromagen. Results indicate that antigen retrieval is necessary to distinguish specific over nonspecific labeling when using the monoclonal antibody on mouse tissue. Mitral, tufted, and granule cells, and their proximal dendrites, were labeled. Granule cell dendrites were clearly visible extending into the EPL. Periglomerular cell perikarya were immunopositive without microwaving, but were barely visible above background with microwaving. Glomeruli contained labeled thin and thick processes. Olfactory nerve axons were negative, but ensheathing cells and their processes were immunopositive. Small, oblong cells of the subventricular layer were strongly labeled. Protoplasmic astrocytes were not visible in the neuropil, but presumptive fibrous astrocytes were labeled in the corpus callosum and hippocampal fornix. Current work concerns localizing NR1 expression at the ultrastructural level. The cellular expression of NR1 suggests a role in neuronal, and perhaps glial, signaling in all bulb layers. Supported by an Alabama Food Animal Health and Disease Research grant.

205 Poster : Neurotransmitters in the Olfactory CNS

A DIVERSITY OF FUNCTIONAL IONOTROPIC GLUTAMATE RECEPTORS ON THE MITRAL CELL SOMATODENDRITIC MEMBRANE

Lowe G.[1] [1]*Monell Chemical Senses Center, Philadelphia, Pennsylvania*

It is well known that the soma and basal dendrites of olfactory bulb mitral cells receive GABAergic inhibition from granule cells mediating lateral and feedback inhibition. It was also known since 1982 that blocking GABA receptors unmasks mitral cell self excitation mediated by somatodendritic glutamate autoreceptors. However, it is widely believed that the mitral soma and basal dendrites are devoid of fast excitatory synaptic inputs, and a recent study by Didier et. al. (2001) proposing excitatory synapses mediated by a novel AP5-resistant NMDA receptor has received little attention. Our previous results from focal uncaging of glutamate on the mitral cell were inconclusive regarding the existence of such NMDA receptors. However, we now report that wide-field laser photolysis reveals a slow current mediated by an AP5-resistant NMDA receptor, exhibiting strong outward rectification in 1.3 mM Mg^{2+}, and block by 15 μM 5,7-dichlorokynurenate. It appears to operate in parallel, not in series, with high affinity AP5-sensitive NMDA receptors also expressed on the mitral cell membrane. Moreover, these receptors coexist with fast AMPA receptors, potentiated by cyclothiazide and blocked by the AMPA-specific antagonist SYM2206, as well as fast kainate receptors. With millisecond activation times and rapid desensitization, the AMPA and kainate receptors are well adapted for the task of fast excitatory transmission as opposed to slow autoexcitation or spillover transmission. These results are consistent with a modified model of conventional fast glutamatergic transmission on the mitral soma and proximal dendrites. Supported by RO1 NIH-DC04208-02.

206 Poster : Neurotransmitters in the Olfactory CNS

THE EFFICACY OF SYNAPTIC TRANSMISSION IN THE OLFACTORY BULB: RELATION TO AMPA RECEPTOR DIVERSITY AND MODULATION.

Blakemore L.J.[1], Trombley P.Q.[1] [1]*Biological Science, Florida State University, Tallahassee, FL*

The kinetics of AMPA receptors influence glomerular synchronicity and reciprocal inhibition in the olfactory bulb (OB). This suggests that cell- and synapse-specific targeting of AMPA receptors with different desensitization properties may facilitate odor-information processing. We recently found that zinc, which is highly concentrated in the OB, can modulate AMPA-evoked currents in the OB. In this study, we used cyclothiazide (CZ), a compound that inhibits AMPA receptor desensitization, to further explore this modulation and investigate kinetic diversity among OB neurons. AMPA (50 μM) evoked currents in all cells examined, but the desensitization kinetics significantly differed between mitral/tufted (M/T) cells and interneurons. Pretreatment with CZ potentiated these currents to highly varying degrees. Zinc (100 μM) also potentiated AMPA-evoked currents in a subset of cells as well as some AMPA receptor-mediated synaptic events in spontaneously active neurons. Pretreatment with CZ (100 μM) reduced or eliminated this effect in most cells, suggesting that zinc's potentiating effect is due to a reduction in AMPA receptor desensitization. Furthermore, zinc had voltage-dependent effects on transient (but not sustained) outward potassium currents, which appeared to alter the timing of action potential generation in response to a long depolarizing current step. Collectively, these results suggest that AMPA receptor desensitization during synaptic transmission, as well as its modulation by zinc, may alter the amplitude, duration, and timing of synaptic events, thus, synaptic efficacy. NIH/NIDCD

207 Poster : Neurotransmitters in the Olfactory CNS

ACTION POTENTIALS FACILITATE COMMUNICATION BETWEEN PROXIMAL AND DISTAL DENDRITIC COMPARTMENTS IN OLFACTORY BULB GRANULE CELLS

Tia S.[1], Strowbridge B.W.[1] *[1]Dept of Neurosciences, Case Western Reserve University, Cleveland, OH*

We used whole-cell recording to define the effects of proximal excitatory inputs to GABAergic granule cells in acute rat olfactory bulb slices. We found that weak depolarizing stimuli injected into the cell body elicits Ca accumulations in the cell body and proximal dendrites (mean amplitude in soma = 5.1 ± .5 % ΔF/F; n = 27; granule cells loaded with 150 μM Oregon Green 1 dextran through the patch pipette). Subthreshold Ca responses appeared to be mediated largely by high-voltage gated Ca channels since they were reduced by bath application of ω-conotoxin MVIIC (5 μM; to 39.3 % of control) and were associated with small regenerative voltage responses. Slightly larger current pulses triggered single action potentials and elicited larger Ca accumulations (11.1 ± 1.1 % ΔF/F; n = 27). Using Ca imaging we found that subthreshold Ca responses were largely localized to the cell body and proximal dendrites. By contrast, Ca accumulations evoked by suprathreshold depolarizing stimuli were widespread and could be observed in distal dendrites. When Na channels were blocked using with bath application of tetrodotoxin (1 μM) or intracellular perfusion with QX-314 (5 mM), small-amplitude Ca spikes could be recorded in granule cell somata. Bath application of 4-AP (100 μM) greatly enhanced the amplitude of these Ca spikes suggesting that proximal K channels regulate the interaction between proximal and distal dendritic compartments. Our results suggest that proximal excitatory synaptic inputs that evoke action potentials in granule cells should be best able to influence distal dendritic functions such as dendrodendritic inhibition. Supported by NIH grant DC04285.

208 Poster : Neurotransmitters in the Olfactory CNS

ANTENNAL LOBE NEURONS USE NITRIC OXIDE FOR PROCESSING OLFACTORY INPUT IN THE MOTH *MANDUCA SEXTA*

Wilson C.[1], Christensen T.A.[1], Nighorn A.[1] *[1]ARL Division of Neurobiology, University of Arizona, Tucson, Arizona*

Nitric oxide (NO) has been implicated in a variety of physiological processes, including the modulation of neural networks in olfaction. The expression patterns of both nitric oxide synthase (NOS) and soluble guanylyl cyclase (sGC) vary between species but generally suggest a neuromodulatory role for NO. Previous experiments in *Manduca sexta* have indicated that NOS is expressed in the axons of olfactory receptor neurons (ORNs), and sGC is expressed in a subset of antennal lobe (AL) neurons that includes GABAergic interneurons, 5HT-immunoreactive interneurons, and projection neurons. This expression pattern suggests that NO may be produced upon odor stimulation of the ORNs, thereby modulating the subsequent response of AL neurons. In order to test this hypothesis, intracellular recordings from AL neurons were conducted while stimulating the antennal nerve with odor or electric shock. NO levels were then manipulated by applying NO signaling inhibitors to the AL. The results indicate that interference with NO signaling has profound effects on the ability of recorded neurons to respond to antennal nerve stimulation. Successfully recorded neurons were also filled with Lucifer yellow and double-labeled with a sGC-α1 antibody. Interestingly, some neurons respond to changes in NO levels but are not sGC-immunoreactive. These results indicate that sGC-containing neurons may be acting on non-sGC-containing neurons to modulate antennal nerve output. Alternatively, NO may be modulating these neurons through a different mechanism, such as S-nitrosylation. Supported by NIH-NIDCD DC04292α

209 Poster : Neurotransmitters in the Olfactory CNS

MOLECULAR ANALYSIS OF THE CALCIUM-DEPENDENT REGULATION OF CGMP FORMATION IN THE ANTENNAL LOBE OF *MANDUCA SEXTA*.

Collmann C.[1], Nighorn A.[1] *[1]Neurobiology, University of Arizona, Tucson, AZ*

Changes in the levels of the intracellular messenger cGMP are thought to play an important neuromodulatory role in the antennal lobe (AL) of *Manduca sexta*. Intracellular calcium levels can affect cGMP formation in AL neurons in two ways. Calcium increases in olfactory receptor neuron axons can activate nitric oxide synthase, triggering nitric oxide (NO) production and the subsequent activation of soluble guanylyl cyclase (sGC) in AL neurons. Calcium can also affect the activities of receptor guanylyl cyclases (rGCs) in AL neurons through the actions of neuronal calcium sensors (NCSs). We have used immmunocytochemistry, in-situ hybridization, and molecular cloning methods to investigate the importance of calcium-regulated changes in cGMP levels in the AL. We find that sGC is expressed in a subset of AL neurons that includes GABA-immunoreactive interneurons, 5-HT-immunoreactive neurons, and projection neurons. We also find that AL neurons express two NCSs: MsNCS-II, which appears to be a homolog of Neurocalcin, and MsFrequenin, which appears to be a homolog of Frequenin. In-situ hybridization shows that both are expressed throughout the AL. In-situ hybridization and immunocytochemistry show that a variety of NO-insensitive GCs are also expressed in AL neurons. These results suggest that NO regulation of cGMP levels is important for a defined subset of AL neurons and that calcium regulation of GC activity through NCSs is important for many AL neurons. Calcium-dependent regulation of cGMP in each AL neuron is likely to be determined by which GCs are expressed. Supported by NIH-NIDCD DC04292

210 Poster : Neurotransmitters in the Olfactory CNS

CHARACTERIZATION OF AN IMMORTALIZED PUTATIVE DOPAMINERGIC (DA) OLFACTORY BULB (OB) CELL LINE.

Saino S.[1], Son J.H.[2], Chun H.S.[2], Baker H.[2] *[1]Dept. Anatomy, Yamagata University, Yamagata, JAPAN; [2]Burke Med. Res. Inst., Cornell University, White Plains, New York*

An immortalized cell line was clonally generated to investigate molecular mechanisms underlying derivation of OB DA interneurons. Cells were taken from 2-day-old mouse pups expressing a 9.0kb tyrosine hydroxylase (TH) promoter/temperature sensitive SV40 Large T antigen (SV40Tag) transgene. TH is the enzyme that catalyzes the first step in DA biosynthesis. Cells cultured at the permissive temperature (33°C) in 10 % fetal bovine serum (FBS)/RF medium had a fibroblast-like appearance. Under differentiation conditions (38.5°C in 0.5 % FBS/RF medium for 5-9 days) cells extended processes resulting in a more neuron-like morphology. At 33°C, neuronal phenotype was established by Western blot detection of the neuronal markers, class III β-tubulin, GAP-43 and neuron specific enolase. After differentiation, SV40Tag expression decreased and levels of neuronal marker proteins were elevated. Glutamic acid decarboxylase protein, a GABAergic marker in OB, was weakly expressed under both culture conditions. Although TH protein was at the level of detection as found in migrating progenitors in vivo, RT-PCR analysis indicated that differentiation conditions increased TH gene transcription. Under differentiation conditions, mRNA levels increased for the transcription factors, Nurr1, NGFI-B and Dlx-2, the growth factors, BMP4 and FGF8 and the Shh receptor, Smo. These data indicate that this cell line is a suitable model system to analyze DA neuron phenotypic differentiation. Supported by NIH grant # AG09686.

211 Poster : Neurotransmitters in the Olfactory CNS

NEUROINHIBITORY ACTIONS OF TAURINE IN THE MAIN OLFACTORY BULB

Kratskin I.[1], Benedusi M.[2], Belluzzi O.[2] [1]*Smell and Taste Center, Univ. Pennsylvania, Philadelphia* [2] *Biology, University of Ferrara, Italy*

The amino acid taurine is abundant in the olfactory bulb (OB), exceeding glutamate and GABA in concentration. In whole-cell patch-clamp recordings in slices of rat OB, we studied the actions of taurine on the principal neurons (PNs), mitral and tufted cells, and the local interneurons, periglomerular (PG) cells. Taurine decreased, in a dose-dependent manner (EC_{50}=2.2 mM), the input resistance of PNs and shifted membrane potential towards E_{Cl}. The $GABA_A$ receptor antagonists, bicuculline and picrotoxin, but not $GABA_B$ receptor antagonists, CGP 35348 and CGP 55845A, blocked the taurine effects. This implies that taurine inhibits PNs by increasing $GABA_A$ receptor Cl^- conductance. PG cells, which also express $GABA_A$ receptors, were insensitive to taurine. Olfactory nerve stimulation evoked monosynaptic excitatory responses in PNs and PG cells voltage clamped at E_{Cl} or treated with picrotoxin. Taurine (5 mM) and the $GABA_B$ receptor agonist baclofen suppressed PNs responses. CGP 55845A, but not bicuculline and the postsynaptic $GABA_B$ receptor antagonist CGP 35348, abolished this suppression. The taurine action most likely was due to $GABA_B$ receptor-mediated inhibition of presynaptic glutamate release. Neither taurine nor baclofen affected PG cell responses. The results suggest that taurine reduces the excitability of PNs and their sensory input without influencing PG cells. Selective inhibitory actions of taurine in the OB may represent a physiologic mechanism protecting PNs from hyperexcitation. Supported by NIH grant DC04083 (IK) and Fondazione Caricento (OB).

212 Poster : Neurotransmitters in the Olfactory CNS

PUTATIVE GABAERGIC OLFACTORY JUXTAGLOMERULAR CELLS FROM GAD-GFP TRANSGENIC MICE HAVE PROPERTIES SIMILAR TO PERIGLOMERULAR CELLS.

Shao Z.[1], Hayar A.M.[1], Puche A.C.[1], Erdelyi F.[2], Szabo G.[2], Shipley M.T.[1] [1]*Dept. of Anatomy and Neurobiology, University of Maryland at Baltimore, MD;* [2]*Dept. of Gene Technology and Developmental Neurobiology, Institute of Experimental Medicine, Budapest, Hungary*

Juxtaglomerular (JG) neurons play important roles in olfactory coding. Though often thought to be local inhibitory interneurons, JG cells, in fact comprise at least 4 different populations: External tufted (ET) cells, short axon (SA) cells and periglomerular (PG) cells of which there are two distinct subtypes: Classic PG cells and 'Hairy' PG cells. Although many JG cells contain GABA, it is not known which JG cell types are GABAergic. We are investigating the morphological and electrophysiological characteristics of JG cells in transgenic mice in which the regulatory region of the gene coding for the GABA synthetic enzyme GAD-65 drives the expression of Green Fluorescent Protein (GFP). In olfactory bulb slices, GAD-65 expressing JG cells express very high levels of GFP and are easily visualized.GFP cells are recorded with the patch-clamp technique and filled with biocytin for subsequent morphological reconstruction. The electrophysiological properties of the cells are characterized in current- and voltage-clamp mode. Most of these cells (14 of 19) exhibit bursts of spontaneous excitatory postsynaptic potentials (EPSPs). Olfactory nerve stimulation evoked bursts of EPSPs with variable latencies in 8 cells tested. Preliminary results indicate that the physiological properties of GAD-65 GFP cells are similar to those of PG and possibly SA cells in rat. The morphological properties of biocytin-labeled cells are currently being analyzed. PHS grants: DC02173, DC00347 & NS36940.

213 Poster : Neurotransmitters in the Olfactory CNS

TASK-DEPENDENT CHOLINE CONCENTRATION IN PRIMARY OLFACTORY CORTEX OF THE HUMAN

Zelano C.M.[1], Kaiser L.[1], Mainland J.D.[1], Khan R.M.[1], Johnson B.N.[1], Bensafi M.[2], Sobel N.[1] [1]*Biophysics, University of California, Berkeley;* [2]*Neuroscience, University of California, Berkeley*

The neurotransmitter acetylcholine (ACh) is thought to aid in learning by suppressing existent intrinsic, not afferent neuronal connections during learning of new memories. In models of learning and recall, this suppression of intrinsic transmission prevents the recall of previously learned memories from interfering with learning of new information. At the same time, ACh decreases the adaptation of pyramidal cells, increasing the response to afferent input. These models suggest that ACh creates an atmosphere conducive to learning new information, while decreased ACh function sets the appropriate dynamics for recall. To estimate levels of ACh in primary olfactory cortex during an odor memory task we used magnetic resonance spectroscopy (MRS), a noninvasive technique to measure concentrations of metabolites in the brain. Choline, a metabolite of ACh, is readily detected by MRS. The scan consisted of a 120s no-odor baseline followed by 240s of odor memory task followed by an additional 60s no-odor baseline. In the task subjects were instructed to learn the appearance order of 5 consecutively presented odorants that repeated 4 times. We used a 4T MR scanner (TE=30ms, TR=1s) to record from an 8 cm3 voxel containing primary olfactory cortex. Pyrolytic graphite was used to increase the homogeneity of the magnetic field. In the one subject scanned to date there was a significant decrease in concentration of choline during the task (P= 0.019). Whereas this first measurement of neurometabolites in human olfactory cortex suggests acetylcholine is modulated in human primary olfactory cortex during an olfactory memory task, a larger sample will be scanned in order to address the validity of this preliminary result. Funding: NIH NIDCD

214 Poster : Neural Plasticity & Regeneration

RECOVERY OF THE P2 ODORANT RECEPTOR SUBTYPE AFTER NERVE TRANSECTION

Yoshida-Matsuoka J.[1], Tsukatani T.[2], Matsuoka M.[1], Costanzo R.M.[1] [1]*Physiology, Virginia Commonwealth University, Richmond, Virginia;* [2]*Otorhinolaryngology, Kanazawa University, Kanazawa, Ishikawa, Japan*

Olfactory neurons express approximately one thousand different receptor subtypes and are continuously replaced within the olfactory epithelium. Although the time course of receptor cell replacement has been studied, little is known about the restoration of odorant receptor subtype expression. In this study, we used a transgenic mouse (P2-IRES-tau-LacZ) to investigate the development and expression of the P2 odorant receptor subtype during recovery from nerve transection. We used GAP-43 and X-gal staining to identify immature neurons and those expressing the P2 receptor subtype. At recovery day 15, the number of P2 neurons reached 20% of control, and most of them (> 65 %) were GAP-43 positive immature neurons. At recovery day 35, the number of P2 neurons increased to 30% of control and a smaller number (< 40 %) were GAP-43 positive. Previous studies have shown that the number of olfactory receptor cells recovers to 60-70% of control levels by day 15 and behavioral recovery is observed at day 40. Our results suggest that the time period required for maturation of P2 odorant receptor neurons may be longer than previously expected. Supported by NIH DC00165.

215 Poster : Neural Plasticity & Regeneration

A MORPHOLOGICAL AND HISTOCHEMICAL STUDY OF THE RESPONSE TO PERIPHERAL DEAFFERENTATION IN THE ADULT ZEBRAFISH OLFACTORY BULB

Heard B.R.[1], Byrd C.A.[1] [1]*Biological Sciences, Western Michigan University, Kalamazoo, MI*

Prior research has shown that following ablation of the olfactory organ in adult zebrafish the olfactory bulb exhibits specific temporal changes in size, laminar structure, and nuclear density (Byrd 2000 Brain Res 866:92). This research investigates the deafferentation-induced changes in the cellular morphology of the adult zebrafish olfactory bulb at various time intervals in an attempt to understand the bulbar response to peripheral injury. Permanent destruction of the right olfactory organ was performed on deeply anaesthetized adult zebrafish. Fish were allowed to survive from 1 hour to 6 weeks. Paraffin-embedded semi-serial sections were stained with hematoxylin and eosin (H&E) to show morphology and with monocyte markers alpha-naphthyl acetate esterase (ANAE) or alpha-naphthyl butyrate esterase (ANBE). At 1, 6, and 24 hours following deafferentation H&E staining reveals no discernable difference among the cellular composition of the deafferented bulb and control bulbs. Low levels of ANAE and ANBE are present normally in the bulb and at early time points there is no significant increase in these labels following injury. Preliminary H&E staining results indicate that 1 week following deafferentation changes are evident in the cellular population in both bulbs. Both H&E and histochemical staining results suggest that the zebrafish olfactory bulb does not mount a significant immune response immediately following deafferentation. This project was supported by NIH-DC04262-01A1.

216 Poster : Neural Plasticity & Regeneration

RECOVERY OF GURMARIN- AND AMILORIDE-SENSITIVITIES OF THE MOUSE CHORDA TYMPANI NERVE AFTER THE NERVE CRUSH AND REGENERATION

Yasumatsu K.[1], Kojima H.[1], Yokota Y.[1], Ninomiya Y.[1] [1]*Oral Neuroscience, Kyushu University, Fukuoka, Japan*

Gurmarin (G) is known to be a peptide that selectively inhibits responses to sucrose in the chorda tympani (CT) in C57BL mice. The peptide, however, suppresses maximally only about half of the response, indicating existence of both G-sensitive (GS) and -insensitive (GI) response components to sucrose in the CT and thereby corresponding GS and GI receptor systems. In the present study, to investigate formation of the two different sucrose response systems, we examined recovery of these components after the CT nerve crush and regeneration in C57BL mice. We compared it with that of NaCl responses and their inhibtion by amiloride. At about 2 weeks after the nerve crush, no significant responses to taste stimuli were observed in the CT. At about 3 weeks after the crush, responses to sucrose and NaCl reappeared. In almost all cases, NaCl responses were not inhibited by amiloride (amiloride-insensitive: AI), whereas in some but not all cases sucrose responses were suppressed by gurmarin (GS). At about 4 weeks after crush, NaCl responses appeared to be amiloride-sensitive (AS). After more than a month, the CT showed sensitivities either to gurmarin or amiloride comparable with those shown by intact animals. These preliminary data suggest that time needed for recovery may not largely different between GS and GI components for sucrose responses, which is considerably different from the case for NaCl responses where AI system recovered much earlier than AS system.

217 Poster : Neural Plasticity & Regeneration

UNILATERAL CHORDA TYMPANI NERVE SECTION INDUCES A BILATERAL INCREASE IN LINGUAL MACROPHAGES IN CONTROL-FED BUT NOT NA+-RESTRICTED RATS.

McCluskey L.P.[1] [1]*Physiology, Medical College of Georgia, Augusta, GA*

Dietary Na+ restriction, in combination with unilateral chorda tympani (CT) nerve section, leads to a rapid decrease in neurophysiological responses to Na+ in the intact CT (Hill and Phillips, 1994). It was proposed that dietary Na+ restriction causes these early functional deficits by preventing beneficial immune activity after denervation. Indeed, upregulation of immune function after unilateral CT section induced recovery of normal Na+ taste function in the Na+-restricted rat (Phillips and Hill, 1996). It was hypothesized in the current study that macrophages—which affect other degenerating neural and epithelial cells—might contribute to this functional plasticity. Adult rats received unilateral CT section followed by dietary Na+ restriction or maintenance on a control diet. Additional groups received sham CT sectioning. Rats were then sacrificed at day 2, 5, 7, or 50 post-sectioning, and cryosections of tongue stained with the ED1 antibody to detect macrophages. In control-fed rats, there was a significant increase in the number of macrophages present on both the cut and uncut sides of the tongue at day 2-7 post-section. In contrast, macrophages did not increase above baseline on either side of the tongue in Na+-restricted rats at any time post-section. Likewise, very few macrophages were present in the tongues of normal control or sham-sectioned control rats. Macrophages are predicted to enter the tongue in response to CT and taste receptor cell degeneration, become activated, and secrete cytokines which maintain normal taste function after injury.

218 Poster : Neural Plasticity & Regeneration

NERVE CUT INDUCED DECREASE OF CHORDA TYMPANI TERMINAL FIELDS IN THE NTS OF ADULT CONTROL AND SODIUM-RESTRICTED RATS.

Cheon B.K.[1], Hill D.L.[2] [1]*Cognitive Psych, University of Virginia, Charlottesville, Virginia;* [2]*Psychology, University of Virginia, Charlottesville, Virginia*

Unilateral section of the chorda tympani nerve (CT) in adult rats results in a mismatch between the size of taste buds and the number of innervating neurons after the nerve regenerates. This mismatch occurs in both rats fed a sodium-replete and a sodium-restricted diet, although the effect is greater in restricted rats. A portion of the mismatch is due to a 20% injury-related decrease in CT axons. Given the large-scale effects in the distal portion of the CT and its target (taste buds), we sought to examine the potential nerve-section induced effects on the central portion. Adult rats received unilateral CT section in the neck and then either placed on a sodium-restricted diet or maintained on a sodium-replete diet. At either 14 or 50+ days post nerve section, both the sectioned and uncut CT were labeled with an anterograde tracer in the tympanic bulla. Rats were sacrificed 24 hours later and terminal fields in the NTS were visualized with a confocal laser microscope. As noted previously in hamster by Whitehead, Barry and colleagues, there is degeneration on the nerve-damaged side. We found an approximate 67% volume reduction compared to the intact side in rats surviving 14 and 50+ days, suggesting that the effects are profound and that reinnervation of taste buds by 40 days does not restore terminal field size. Preliminary evidence suggests that there are no diet-related differences in amount of degeneration. Current work focuses on earlier post nerve section periods and a determination where the majority of degeneration occurs. Supported by NIH grant DC0376.

219 Symposium : AChemS-25 Anniversary Symposium: Perspectives on the Chemical Senses

OLFACTION OVER 25 YEARS: HOW HAVE THINGS CHANGED, WHAT MIGHT THEY TELL US?

Kauer J.S.[1] [1]*Neuroscience, Tufts University, Boston, Massachusetts*

Despite the enormous advances of the last 25 years, it is still not clear how the olfactory system detects and identifies odors at vanishingly low concentrations. There have been great improvements in understanding structural and functional elements that extend from the molecular to systems levels, but exactly how we perceive the odor of coffee or the fragrance of jasmine is an enduring neurobiological problem. Continuing work on the anatomy, biochemistry, physiology, molecular biology, and behavior of olfaction seeks to understand this system that intersects with disparate aspects of life that range from the life-determining to the hedonistic. With the mass of new information, it is a propitious time to ask to what degree might olfaction inform the study of other brain systems. Thus, rather than looking to other sensory modalities for clues as to how olfaction works, we can begin to examine how might the sense of smell provide fresh insight into other regions of the CNS. There are many new data on olfactory function that will likely yield insight into other brain regions: why are there so many olfactory receptor genes (do they, in fact, also relate to other functions)?; what underlies the wide discrimination and deep sensitivity for molecular structure (individual receptors - emergent circuit functions)?; what is the basis for the system's resilience to damage; how is stable function maintained in the face of cellular turnover?; how are complex societal behaviors shaped by odors and how are odors shaped by behavior?; how are the basic aspects of disseminated and redundant function used in other systems?; and what aspects of olfaction's ancient phylogenetic origins have contributed to generalized functional principles?

220 Symposium : AChemS-25 Anniversary Symposium: Perspectives on the Chemical Senses

TWENTY-FIVE YEARS OF DEFINING TASTE SPECIFICITY

Frank M.E.[1], Hettinger T.P.[1] [1]*Neurosciences, Dept. Oral Diagnosis, UConn Health Center, Farmington, CT*

At *AChemS I* we recognized the taste qualities sweet, *umami*, salty, sour and bitter as we do now in humans, but the categorizations are becoming less clear as we examine other species' taste systems. We debated receptor and neuron specificity and this issue remains with us, although we now formulate the problem differently as we can relate chemicals to molecular receptors, second messengers and cellular physiology as well as neuronal responses and taste perception. Recent studies define a close correspondence between identified molecular receptors and specific taste qualities. We were concerned that peripheral and central neurons were not tuned to a single taste quality. Now we know this imperfect reductionism was based, in part, on simplifications such as equating a chemical stimulus with a single qualitative taste attribute or equating an oral chemo-stimulus with a taste. We entertained a stimulus dimension analogous to frequency, but taste chemistry must be based on discrete categories of functional groups, stereochemistry, and general features such as polarity and size. And, although we knew of species differences, we now appreciate better the genetic and evolutionary diversity of gustation and food selection. Finally, although we assume that specific molecular receptors define perceptual stimulus quality, we find taste stimuli have both specific and distributed representations in the gustatory pathways. Combining functional neuroimaging and psychophysics may identify the various coded attributes of taste stimuli. [Supported by NIH: R01 DC04099]

221 Symposium : AChemS-25 Anniversary Symposium: Perspectives on the Chemical Senses

25 YEARS OF SOCIAL COMMUNICATION: THE RELEVANCE OF BEHAVIORAL STUDIES TO ADVANCES IN THE CHEMICAL SENSES.

Vickers N.J.[1] [1]*Biology, University of Utah, Salt Lake City, UT*

During the past 25 years, AChemS has benefited greatly from being a relatively small, tight-knit community in which social interactions have played an important role in shaping the society. Similarly, social communication and interactions between animals mediated by the chemical senses have provided a profitable framework within which to understand how these senses are organized to detect, process, and discriminate the complexities of the chemical world that surrounds them. Insect and other invertebrate systems can be included amongst those that have continued to evoke interest throughout the 25 year existence of AChemS. The longevity of our curiosity in these systems is in large part attributable to advances in our understanding of the behavior of these animals that in turn has provided an appropriate context for neurophysiological and molecular investigations. Such studies have been facilitated by a number of factors including the availability of a large number of species often with documented phylogenetic relationships, the ability to identify behaviorally relevant chemicals, the accessibility of peripheral and central structures dedicated to detection and processing of these chemicals, and of course, the fascinating array of animal behaviors and interactions mediated by odorants/tastants. In this talk I will consider how the interactions between these various factors have contributed to advances in our understanding of the chemical senses over the past quarter century.

222 Slide : Olfactory Function in Health & Disease

MODELING NASAL AIRFLOW AND ODORANT TRANSPORT: IMPLICATIONS FOR ODOR PERCEPTION

Zhao K.[1], Scherer P.W.[1], Hajiloo S.A., Dalton P.[3] [1]*Bioengineering, University of Pennsylvania, Philadelphia, PA;* [3]*Monell Chemical Senses Center, Philadelphia, PA*

Recent studies comparing CT or MRI images of an individual's nasal anatomy with measures of their olfactory sensitivity have found a strong correlation between specific anatomical areas and performance on olfactory assessments (Hornung 2001, Damm 2002, Leopold 1988). Using computational fluid dynamics (CFD) techniques, we have developed a method to quickly (< few days) convert nasal C-T scans from an individual patient into an anatomically accurate 3-D numerical nasal model that can be used to predict airflow and odorant transport and ultimately, olfactory sensitivity. The 3-D model can be also be rapidly modified to depict various anatomical deviations, such as polyps and their removal, that may alter nasal airflow and impair olfactory ability. To evaluate the degree to which variations in critical nasal areas such as the olfactory slit and nasal valve can alter airflow and odorant transport, we simulated inspiratory and expiratory airflow with odorants using numerical finite volume methods. Results suggest that anatomical changes in the olfactory region (upper meatus below the cribriform plate) and the nasal valve region will strongly affect airflow patterns and odorant transport through the olfactory region, with subsequent effects on olfactory perception. The ability to model odorant transport through individualized models of nasal passages holds significant promise for relating anatomical deviations to generalized or selective disturbances in olfactory perception and may provide important guidance for treatments for nasal-sinus disease, occupational rhinitis and surgical interventions that seek to maximize airflow and improve deficient olfactory function. Supported by NIH P50-DC 00214

223 Slide : Olfactory Function in Health & Disease

FMRI STUDY OF HUMAN OLFACTION AND NORMAL AGING AT HIGH FIELD

Wang J.[1], Eslinger P.J.[2], Smith M.B.[1], Yang Q.X.[1] *[1]Radiology, Pennsylvania State University, Hershey, PA; [2]Neurology, Pennsylvania State University, Hershey, PA*

Functional MRI (fMRI) was used to investigate the effect of aging on human brain activation with olfaction. Eleven young (23.9+/-1.6 yo) and 8 aged (66.4+/-4.4 yo) right-handed healthy subjects received fMRI on a 3T system. The average scores on the University of Pennsylvania Smell Identification Test (UPSIT) were 37 in the young group and 34 in the aged group ($p<0.001$). T2*-weighted EPI images were acquired during the execution of the olfactory stimulation paradigm, which consisted of five cycles of resting (50 sec) and stimulation (25 sec). In the two age groups, significant activations were observed in major known olfactory structures including primary olfactory cortex (POC), amygdala (Amyg), entorhinal cortex, hippocampal structure, orbitofrontal cortex, and insula. In addition, activations were found in middle and inferior frontal gyri, middle temporal gyrus, anterior cingulate, basal ganglia, thalamus, cerebellum, and visual cortex. However, the aged group had significant reduction of overall activations in the olfaction related structures ($p=0.035$), especially in the right POC/Amyg area. The reduction of activation size in the olfactory structures correlated with decline in UPSIT scores of older subjects ($r=0.47$, $p<0.05$). Strong positive correlations were found between UPSIT scores and activation sizes in both right POC/Amyg area and right insula ($r=0.66$ and 0.50, $p<0.05$). Strong correlations were also found between the activation strength and age or UPSIT scores in the bilateral POC/Amyg areas and right insula ($r=0.49\sim0.84$, $p<0.05$). Results indicated that aging-related changes in brain and olfaction can be evaluated with fMRI at high fields. This work is supported by NIH grant (RO1 EB00454-01A1).

224 Slide : Olfactory Function in Health & Disease

ODOR-INDUCED FMRI BRAIN ACTIVATION IN THE HEALTHY ELDERLY AND AD PATIENTS

Tabert M.[1], Albers M.[2], Zarahn E.[1], Zimora D.[1], Lorig T.[3], Small S.[2], Doty R.[4], Devanand D.[1] *[1]Psychiatry, Columbia University, New York, NY; [2]Neurology, Columbia University, New York, NY; [3]Psychology, Washington and Lee University, Lexington, VA; [4]Smell & Taste Center, University of Pennsylvania, Philadelphia, PA*

In Alzheimer's disease (AD), plaques and tangles occur early in the olfactory bulbs, anterior olfactory nucleus, piriform cortex, and entorhinal-hippocampal-subicular complex. AD patients consistently show deficits on psychophysical tests of olfaction compared to controls. Few clinical studies have examined the neural correlates of these deficits. This pilot study examined odorant-induced fMRI activation in 2 early AD patients and 2 controls. We hypothesized that AD patients will show decreased activation in olfactory related regions (e.g., piriform) relative to controls. During fMRI scanning, common odors (e.g., vanilla) were presented via an olfactometer (ON-15 s; OFF-30 s). Subjects indicated the onset of each odor. Scans were analyzed using SPM99. Controls demonstrated widespread activation in olfactory-related brain regions (e.g. piriform, parahippocampal gyrus, hippocampus, cingulate, insula, and amygdala). Activation in AD patients occurred only in the amygdala and piriform, and to a much smaller extent. These preliminary findings suggest that there is a measurable difference in olfactory related brain regions between early AD patients and controls. If the magnitude of the observed differences holds up in a larger sample of subjects, fMRI activation patterns may have clinical applicability as an early index of AD pathology. Funded by Alzheimer's Association Investigator-Initiated grant to Devanand.

225 Slide : Olfactory Function in Health & Disease

PROGNOSIS FOR OLFACTORY DYSFUNCTION: THE GOOD, THE BAD, AND THE UGLY

Pribitkin E.D.[1], Cowart B.J.[2], Rosen D.[1], Klock C.T.[2] *[1]Department of Otolaryngology-Head and Neck Surgery, Thomas Jefferson University, Philadelphia, Pennsylvania; [2]Monell Chemical Senses Center, Philadelphia, Pennsylvania*

Few treatment options are available for patients who suffer from any form of olfactory dysfunction, and none for those whose loss is secondary to sensorineural damage. Nonetheless, the olfactory system is resilient and the neuroepithelium capable of regeneration; thus, there is the potential for spontaneous recovery following loss from a variety of etiologic factors. However, the few longitudinal studies of patients with smell dysfunction have produced conflicting results concerning the likelihood of recovery. At least in part, this likely arises from small sample sizes and considerable variation in the length of time patients had sustained a loss prior to their initial evaluation. We will report on re-test findings from 124 patients who presented to the Monell-Jefferson Taste & Smell Clinic with an olfactory complaint and represent the major etiologic categories of olfactory dysfunction: nasal-sinus disease (NSD), upper-respiratory infection (URI), head trauma (HT) and idiopathic dysfunction (ID). Our findings indicate that, in fact, a relatively high proportion (>50%) of patients with dysfunctions secondary to URI, as well as NSD, show partial or complete recovery. On the other hand, this still leaves many patients without relief, and in the case of URI patients, the recovery process may be quite lengthy. Moreover, recovery rates in HT and ID patients are substantially lower, and olfactory function may actually continue to deteriorate in a fifth of these cases.

226 Slide : Olfactory Function in Health & Disease

APOE-∈4 IS ASSOCIATED WITH INTRUSION ERRORS IN EPISODIC RECOGNITION MEMORY FOR OLFACTORY BUT NOT VISUAL STIMULI

Gilbert P.E.[1], Dean P.[2], Hajducko E.[2], Murphy C.[2] *[1]Head and Neck Surgery, School of Medicine, University of California, San Diego, San Diego, CA; [2]Psychology, San Diego State University, San Diego, California*

Episodic recognition memory for odors, faces, and unfamiliar symbols was assessed in nondemented, apolipoprotein E ∈4 positive individuals and age- and gender-matched ∈4 negative controls. Recognition memory performance of ∈4 positive individuals was comparable to ∈4 negative controls for both olfactory and visual stimuli. However, ∈4 positive individuals committed significantly more false positive errors for olfactory but not visual stimuli compared to ∈4 negative controls. The results suggest that odor memory may be more susceptible to intrusion errors than memory for other types of stimuli in individuals at risk for Alzheimer's disease. Since the ∈4 allele of the ApoE gene has been shown to be an important risk factor for Alzheimer's disease, the data suggest that intrusion errors in recognition memory tasks involving olfactory stimuli may offer insight into the relationship between the early neuropathological changes and behavioral memory deficits associated with the onset of Alzheimer's disease. Supported by NIH grant #AG04085 from the National Institute on Aging to Claire Murphy and training grant #DC00032 to Terence M. Davidson.

227 Slide : Olfactory Function in Health & Disease

MULTIPLE CHEMICAL SUPERSENSITIVITY - THE MECHANISMS OF ACTION

Savic I.[1], Berglund H.[2], Hillert l.[3] *[1]Neuroscience, Karolinska Institute, Stockholm, Sweden; [2]Medicine, stockholm, Sweden; [3]Evironmental Medicine, Karolinska Institute, Stockholm, Sweden*

Multiple chemical supersensitivity (MCS) is a rapidly increasing condition, yet with unknown pathophysiology. To investigate a possible dysfunction in the odour processing neuronal circuits, we conducted PET studies of regional cerebral blood flow (rCBF) during smelling of odorants and odourless air in twelve healthy- and twelve MCS-females. The underlying hypothesis was that odour-induced increase in rCBF in the olfactory brain would, due to neuronal hyper-sensitivity, be higher in MCS- compared to control-subjects. The compounds used were vanillin, acetone, two putative pheromones (4,16-androstadien-3-one and oestra-1,3,5(10),16-tetraen-3-ol), four familiar and four unfamiliar odorants. The study also included on-line measurements of respiratory index (amplitude * frequency), and RR intervals before, and during each PET scan. MCS subjects consistently activated the odour processing brain regions less than controls, despite reported, and physiologically indicated (decreased RR interval) distress. In parallel, they showed an increased, odorant-related, activation of the anterior cingulate cortex and cuneus - areas associated with harm avoidance and recall, respectively. No regional group differences were observed during smelling o odourless air. The results do not support the notion that MCS is associated with neuronal sensitisation. Nonetheless, the reactions seem to have a physiological ground. We propose that this condition may be a consequence of aversive odor-conditioning leading to harm avoidance, and top-down regulation of odor-response, and suggest odour counter-conditioning as a suitable treatment strategy.

228 Slide : Olfactory Function in Health & Disease

EFFECTS OF THE APOE E4 ALLELE ON OLFACTORY FUNCTION IN DOWN SYNDROME

Sliger M.[1], Lander T.[2], Murphy C.[1] *[1]Psychology, San Diego State University, San Diego, CA; [2]Surgery, Head and Neck, UCSD Medical Center, San Diego, CA*

Alzheimer's disease is a progressive neurodegenerative disease that currently affects over 4 million Americans. Recently an increased frequency of the apolipoprotein E e4 allele has been established as a genetic risk factor for AD. In addition, by age 35, the classical neuropathological changes of AD are found in nearly all individuals with Down syndrome (DS) and it is well known that the olfactory processing area is one of the first areas affected in AD. The present study investigated the prevalence of ApoE e4 allele in a well-characterized sample of individuals with DS and the effects of the ApoE e4 allele on olfactory function in DS. Participants were 34 with DS and 34 normal controls. Genomic DNA was prepared from blood samples to obtain ApoE status for the DS participants. Olfactory function was assessed with odor identification. Prevalence of the e4 allele in the DS participants was compared with rates in the general population. Results indicate (1) that participants with DS had significant deficits in olfactory functioning; (2) that within the DS group, participants with the e4 allele had poorer odor identification that those without the e4 allele; and (3) that the frequency of the ApoE e4 allele in the DS participants was not significantly different from that expected from the general population. The results support the hypothesis that individuals with DS who have an additional genetic risk factor for AD, the ApoE e4 allele, exhibit greater deficits in odor identification; and that DS and ApoE status confer independent risks for AD. Supported by NIH grant AG04085 (CM) and training grant DC00032 (TL).

229 Slide : Olfactory Function in Health & Disease

DIFFERENTIAL LOSS OF RETRONASAL RELATIVE TO ORTHONASAL OLFACTION IN A CLINICAL POPULATION

Cowart B.J.[1], Halpern B.P.[2], Rosen D.[3], Klock C.T.[1], Pribitkin E.D.[3] *[1]Monell Chemical Senses Center, Philadelphia, Pennsylvania; [2]Neurobiology and Behavior, Cornell University, Ithaca, New York; [3]Department of Otolaryngology-Head and Neck Surgery, Thomas Jefferson University, Philadelphia, Pennsylvania*

A substantial minority of patients who present with smell and/or food flavor complaints do not evidence loss on traditional clinical measures of orthonasal (ON) olfactory function, or the degree of loss reflected in their test scores seems to be substantially less than that being reported. To determine if this anomaly might in part reflect differential loss of retronasal (RN) olfactory function, we developed a simple clinical test of RN odor identification. Two RN presentations of each of 5 flavor extracts are intermixed with 2 ON ones in order to compare performance in the two presentation modes. 194 non-anosmic patients and 50 normal controls have been tested. Patients scored significantly lower than controls on both RN and ON presentations, but also showed significantly greater discrepancies between RN and ON scores. 13.4% of patients evidenced a profound RN deficit but scored at least 4 points higher on ON presentations. Half of these would have been classified as normal or only borderline hyposmic based on their performance on our standard clinical test battery. This marked discrepancy in RN and ON abilities was observed most often in patients with ongoing nasal-sinus disease. These findings indicate that, at least in a clinical population, RN function does not necessarily parallel ON function, and specific RN deficits may underlie some patient complaints. Supported by NIH grant P50 DC20014.

230 Symposium : Patterning in Olfactory Systems: How Much is Pre-specified?

PATTERNING IN OLFACTORY SYSTEMS: HOW MUCH IS "PRESPECIFIED?"

Tolbert L.[1] *[1]University of Arizona, Tucson, AZ*

During development of the olfactory system, which features are "prespecified" and which rely most heavily on cellular interactions with their environments? One might go further and ask: what exactly is meant by "prespecified?" Accumulating evidence from a broad array of species indicates that certain aspects of the development of both the olfactory epithelium and the olfactory bulb (and equivalent structures in invertebrates) develop only when then two structures "speak" to each other; other aspects develop normally even in the absence of interaction between the two structures, suggesting innate specification. Even in those situations that suggest independence of structures, intercellular interactions, both neuron-neuron and neuron-glia, have been found to be essential for key steps in cellular development. In this symposium, Laura Lopez-Mascaraque (Cajal Institute, Spain) will present evidence for more autonomy of the developing mammalian olfactory bulb than was previously appreciated, and James St. John (University of Queensland, Australia) will discuss complementary experiments indicating that the axons of olfactory receptor neurons can sort and converge in the absence of their brain targets. Andrew Chess (MIT) will talk about specification of olfactory receptor neurons and the "interdependence" of receptor axons in maintaining the glomeruli. Reinhard Stocker (University of Fribourg, Switzerland) will present the surprising finding that projection neurons in the fly antennal lobe appear to be specified by their lineage to innervate particular olfactory glomeruli, and will make the case for studying the miniature larval olfactory system as an attractive chemosensory model system. Together, the presentations will challenge and extend current notions of dependence and interdependence of developing olfactory structures.

231 Symposium : Patterning in Olfactory Systems: How Much is Pre-specified?

INTERDEPENDENCE AND COORDINATION OF AXONS AND ODORANT RECEPTOR GENES

Chess A.[1] [1]*Biology, Massachusetts Institute of Technology, Cambridge, MA*

In mice, the odorant receptor is involved in an instructive manner in the axonal convergence to distinct glomeruli but little is known about the maintenance of this convergence. We have analyzed a transgene that contains a tau-lacZ marked odorant receptor gene (M12). The neurons expressing the marked transgenic M12 gene are distinct from the neurons expressing the endogenous gene and project to distinct glomeruli. While convergence is always observed in newborn mice, surprisingly, in adult mice, convergence is not always maintained. Moreover, we observe a positive correlation between the number of neurons expressing the transgenic receptor and the probability of maintaining convergence. These observations, taken together with the variability observed in wild-type and genetically manipulated mice suggest that olfactory neurons are interdependent; they require the presence of other similar axons to maintain a glomerulus. Genome-wide aspects of the regulation of odorant receptor genes will also be discussed.

232 Symposium : Patterning in Olfactory Systems: How Much is Pre-specified?

THE SIMPLE BUT VERTEBRATE-LIKE ADULT OLFACTORY SYSTEM OF DROSOPHILA DERIVES FROM A MINIATURE LARVAL SYSTEM WITH NO MORE THAN 21 RECEPTOR NEURONS

Stocker R.F.[1], Python F.[1], Jefferis G.[2], Marin E.[2], Luo L.[2] [1]*Dept. of Biology, University of Fribourg, Fribourg, Switzerland;* [2]*Dept. of Biol. Sciences, Stanford University, Stanford, CA*

There is emerging evidence that vertebrate and insect olfactory systems may be organized according to common principles, though differing in cell numbers. This is why *Drosophila* with merely 1300 odorant receptor neurons (ORNs) has become a focus in chemosensory research. Surprisingly, larvae include no more than 21 ORNs, suggesting an even simpler olfactory model system. Yet, is this drastic reduction of ORNs accompanied by comparable simplicity at the central level? Single cell labeling using the flip-out technique (Wong et al., Cell 109, 229, 2002) shows that the arborizations of afferent fibers and projection neurons (PNs) recognize subregions of the larval antennal lobe, similar to adult glomeruli. Hence, the central organization of the system does not seem to be less complex than in the adult. In contrast, the sensory level is not only drastically reduced in terms of neuron numbers, but organized in an essentially different way, showing for example striking links between smell and taste. During metamorphosis, the larval ORN afferents become replaced by adult afferents. The PNs on the other hand persist, which suggests that they may play a crucial role in the reorganization of the antennal lobe, associated with the change in sensory input. In agreement with this notion, clonal analysis demonstrates prespecification of PNs, independent of newly arriving adult afferent fibers. (supported by Swiss National Funds and NIH grants)

233 Symposium : Patterning in Olfactory Systems: How Much is Pre-specified?

PRIMARY OLFACTORY AXONS CAN SORT OUT AND CONVERGE INDEPENDENTLY OF THE OLFACTORY BULB IN MOUSE

St John J.A.[1], Key B.[1] [1]*School of Biomedical Sciences, University of Queensland, Brisbane, QLD, Australia*

Primary olfactory axons expressing the same odorant receptor gene sort out from axons expressing unlike receptors and converge to topographically fixed glomerular targets in the olfactory bulb. We have examined the guidance of axons expressing the P2 odorant receptor when they were challenged with different cellular environments in vivo. In extratoes mice the olfactory bulb is lacking and is replaced by a fibrocellular mass devoid of second-order neurons. In these mice primary olfactory axons sort out and converge to form a locus without appropriate targeting. Convergence without targeting also occurred following bulbectomy. Thus, the behaviour of olfactory axons in these different models clearly indicates that sorting and convergence of axons expressing the same odorant receptor occurs independently of the bulb. Moreover these results indicate that convergence and targeting of olfactory axons are separate events mediated by different guidance cues. To better understand how axons interact and sort out we have examined primary olfactory axon behaviour in vitro using high resolution time-lapse microscopy and have found that axon-axon interactions in vitro result in either attraction, repulsion, or no response. These behaviours are consistent with differential sorting of axons in the nerve fibre layer of the olfactory bulb. This work was supported by grants to JStJ and BK from the National Health and Medical Research Council of Australia.

234 Symposium : Patterning in Olfactory Systems: How Much is Pre-specified?

AN OLFACTORY BULB-LIKE STRUCTURE DEVELOPS IN THE ABSENCE OF OLFACTORY SENSORY INPUT IN MOUSE

Lopez-Mascaraque L.[1], Rivera R.[1], García C.[1], Valverde F.[1], De Carlos J.A.[1] [1]*Developmental Neurobiology, Instituto Cajal-CSIC, Madrid, Spain*

The development of the olfactory bulb (OB) depends upon differentiation of a repertoire of several cell populations and the initiation of the central projections. The role of the arrival of the sensory input of the olfactory sensory neurons located at the olfactory epithelium (OE) still remains unclear. Olfactory development and sensory input from the OE may be two independent processes from one another. Our previous data have shown the development of an olfactory bulb-like structure (OBLS), in the Pax-6 homozygote mutant mice, in the absence of an OE (López-Mascaraque et al., 1998). This structure is labelled with specific OB markers and gives rise to a lateral tract, like the lateral olfactory tract in the control mice (Jiménez et al., 2000, López-Mascaraque and De Castro, 2002). Thus, neurogenesis timing, specific OB markers and a lateral tract formation evidences the OBLS as a prospective OB (Jiménez et al., 2000). The aim of the present work is to examine the precise role of the OE in the OB´s formation using different cultures combined with tracer injections and immunocytochemistry in both mutant and control mice.Cocultures of OBLS with OE explants, show a reorientation of the mitral-like dendrites of the OBLS and a glomeruli-like tuft formation, as occurs in the OB of control mice. Thus, we conclude that the initial formation of an OB is not directly dependent on the previous existence of an OE, due to the fact that an OBLS, that behaves just like an OB of a control mouse, develops in the Pax-6 mutant mice. However, the OE is necessary for the reorientation of mitral/tufted dendritic processes and glomeruli-like formation in the OB and OBLS. (Supported by: MCYT, Research grant BFI2002-03554)

235 Poster : Olfactory Receptor Genes

HUMAN SPERM CHEMOTAXIS: FUNCTIONAL ROLE OF A PREVIOUSLY UNDESCRIBED TESTICULAR ODORANT RECEPTOR

Spehr M.[1], Gisselmann G.[1], Poplawski A.[1], Riffell J.A.[2], Wetzel C.H.[1], Zimmer R.K.[2], Hatt H.[1] *[1]Department of Cell Physiology, Ruhr-Universitaet Bochum, Bochum, Germany; [2]Department of Biology, University of California, Los Angeles, California*

Ever since their discovery in 1992, the expression of distinct members of the olfactory receptor (OR) gene family on various spermatozoa has remained functionally mysterious. However, testicular OR expression favors the idea that OR-mediated chemoreception may play an important role in sperm physiology. To shed more light on the functional meaning of OR expression on mammalian spermatozoa, we identified by means of RT-PCR, cloned and functionally expressed in HEK 293 cells a previously undescribed human testicular OR. Using ratiofluometric Ca2+ imaging techniques we characterized the receptor`s molecular receptive range. Those odorants characterized as ligands for the recombinantly expressed testicular receptor also induced Ca2+ signals in head and midpiece of human spermatozoa. For the first time, we were also able to identify an OR antagonist which specifically inhibits the action of different ligands on the recombinant receptor as well as on human spermatozoa. Finally, we present conclusive evidence from computer-assisted video motion analysis (CAVMA) and flat capillary assays for a role of this testicular OR and its ligands in human sperm chemotaxis.

236 Poster : Olfactory Receptor Genes

AN ODORANT RECEPTOR FROM *ANOPHELES GAMBIAE* THAT IS HIGHLY CONSERVED ACROSS INSECT TAXA

Pitts R.J.[1], Fox A.N.[1], Melo A.C.[1], Zwiebel L.J.[2] *[1]Biological Sciences, Vanderbilt University, Nashville, TN; [2]Biological Sciences, Vanderbilt University, Nashville, Tennessee*

Anopheles gambiae is a highly anthropophilic mosquito responsible for the vast majority of malaria transmission in sub-Sahara Africa. The biting behavior of this disease vector is influenced largely by the animals' sense of smell, which is presumed to be facilitated by G protein-coupled receptor signaling as in other model olfactory systems. We have initiated studies intended to elucidate the molecular mechanisms underlying olfaction in *Anopheles gambiae* and we have identified by various methods a number of potential olfactory signal transduction genes, including approximately eighty candidate odorant receptors. One of these genes, *GPRor7*, is extremely well conserved within several Dipterans and across insect orders. By RT-PCR analysis, the gene product is expressed in Anopheles tissues expected to be olfactory in their function and during all stages of the insects' development. Further, the GPRor7 peptide is found, in immuno-stained sections, specifically within many, if not all, antennal olfactory neurons. These results indicate that *GPRor7* is likely not only to be of specific importance to Anopheles olfaction but, indeed, of insect olfaction in general. This work has been funded by the NIH/NIDCD and the WHO/TDR.

237 Poster : Olfactory Receptor Genes

IDENTIFICATION OF CANDIDATE OLFACTORY RECEPTORS OF THE MOTH *HELIOTHIS VIRESCENS*

Krieger J.[1], Raming K.[2], Dewer Y.M.[1], Bette S.[1], Conzelmann S.[1], Breer H.[1] *[1]Institute of Physiology, University of Hohenheim, Stuttgart, Germany; [2]Target Research Insecicides, Bayer Crop Science, Monheim, Germany*

In the antennae of moths, which have been proven as invaluable model for studying principles of odor perception, recognition and processing of olfactory signals is accomplished by antennal sensory neurons located in sensillar hair structures. Pheromones and odorants are supposed to be recognized by seven transmembrane domain receptor proteins in the dendritic membrane of these cells; however, the molecular identity of moth olfactory receptors is still elusive. We have assessed a genome database of the moth Heliothis virescens for sequences which may encode heptahelical receptors and employed exon-specific probes to screen an antennal cDNA library of the moth. Analysis of the isolated cDNA-clones led to the discovery of a divergent gene family encoding putative seven transmembrane domain proteins. The notion that they may encode candidate olfactory receptors of the moth is supported by the finding that several subtypes are specifically expressed in the antennae. Moreover, in situ hybridization experiments revealed that they are indeed expressed in antennal sensory neurons. By means of double-labeling in situ hybridization studies it was demonstrated that each receptor subtype appears to be expressed in a distinct population of sensory cells. This work was supported by the Deutsche Forschungsgemeinschaft and the Bayer AG, Leverkusen.

238 Poster : Olfactory Receptor Genes

A COMPARATIVE STUDY OF OLFACTORY RECEPTORS IN SEA TURTLES, LAND TURTLES, AND ALLIGATOR

Vieyra M.L.[1], Vogt R.G. *[1]Department of Biological Sciences, University of South Carolina, Columbia, SC*

All species of sea turtles are considered threatened or endangered; accidents involving fishing nets or hooks being a leading cause of death to these animals. Since it is sensory information that is drawing these creatures to their deaths it may be sensory repellents that play a role in saving them. It has thus become important to understand how these animals use their various senses. Olfaction has been shown to play a role in finding food, avoiding predators and returning to native beaches. A molecular approach to studying olfaction may give clues as to how widely this sense is used. DNA was isolated from the blood of three species of sea turtle (Caretta caretta, Chelonia mydas, and Dermochelys coriacea) as well as one freshwater turtle (Sternotherus odoratus), one terrestrial turtle (Terrapene carolina) , and alligator. Primers based on conserved regions of olfactory receptors in birds and mammals were used to amplify OR genes using PCR. Genes were then inserted into a plasmid vector, grown up in bacteria and sequenced. A comparison of genes from the different species shows a trend towards functionality as an animal becomes more terrestrial. About 50% of the OR genes from sea turtles and alligator have internal stop codons, less in freshwater turtles (34%), and very few in terrestrial turtles (2%). The actual number of genes amplified from the terrestrial turtle was greater then from the other species as well (about 60% more). These results may indicate a greater use of olfaction in turtles (and perhaps reptiles in general) from a terrestrial habitat. Funding (RVG), NMFS, Honolulu.

239 Poster : Olfactory Receptor Genes

OR37-RECEPTORS: A UNIQUE SUBFAMILY OF OLFACTORY RECEPTORS

Strotmann J.[1], Hoppe R.[1], Conzelmann S.[1], Breer H.[1] *[1]Institute of Physiology, University of Hohenheim, Stuttgart, Germany*

While sensory neurons expressing distinct receptor types are usually distributed within broad rostro-caudal zones, those neurons expressing an OR37 receptor are assembled in a small area of the nasal neuroepithelium. A comparative analysis of the mouse and human genome revealed that in both species OR37 genes are organized in two clusters: cluster-I comprising 5 genes and cluster-II containing 3 genes in mouse and 7 genes in human. The pronounced diversity of non-coding sequences in both genomic loci indicates a long coexistence of the two gene clusters and the genes within the clusters, nevertheless the coding regions showed a remarkably high level of sequence identity; thus indicating that OR37 genes may be under negative selection pressure in contrast to "normal" olfactory receptor genes and suggesting that the OR37 receptors may be tuned to recognize distinct sets of signaling molecules. The axonal projection pattern of OR37 expressing neurons was analyzed using a gene targeting strategy. It was found that each population of neurons expressing one of these highly homologous receptors innervates a distinct glomerulus but all are grouped together within a small focal area of the bulb. Thus, each glomerulus receives input very precisely from only one receptor-specific population of neurons. During development, however, the axonal targeting of neurons expressing highly related mOR37 receptors is largely unfocused until the day of birth; the projection into distinct glomeruli is mainly achieved in a short postnatal period until pn3.

240 Poster : Olfactory Receptor Genes

UNDERSTANDING THE RELATIONSHIP BETWEEN SEQUENCE VARIATION AND ODORANT RECOGNITION IN OLFACTORY RECEPTORS

Schlador M.L.[1], Walker M.[1], Trask B.J.[1] *[1]Div. of Human Biol., Fred Hutchinson Cancer Research Center, Seattle, WA*

The olfactory receptor (OR) family contains approximately 1000 genes arrayed in clusters throughout the genome. The OR family has expanded through a series of gene duplication events followed by diversification and continues to evolve. Many genes have become pseudogenes following primate speciation or during human evolution. In some cases, enhanced sequence variation in ORs appears to be due to positive selection. Unfortunately, almost nothing is known about the effects of sequence variation on odorant recognition by individual ORs. Thus, it is not clear when duplicated genes or orthologs from different species have accumulated enough changes to recognize different odorants. In order to understand the role of interspecies (orthologous) and intraspecies (allelic) sequence variation on ligand recognition by ORs, we have selected five ORs with known ligands for comparative sequencing and functional analysis. We are sequencing these ORs from 50 human individuals, multiple non-human primates, and wild-derived mouse strains. In an initial sampling of four genes from 20-25 human individuals, we found that 15/17 nucleotide substitutions result in an amino acid change. In addition, 25/48 differences between the human and chimp orthologs lead to an amino acid change. These data indicate that the subset of ORs chosen for our analysis contain a high degree of sequence variation. We are currently in the process of establishing functional assays to determine the ligand recognition properties of orthologous and allelic OR variants. Supported by NIH grants DC04209 (BJT) and T32 CA80416-04 (MLS).

241 Poster : Olfactory Receptor Genes

EXPRESSION OF RAT ODORANT RECEPTOR mRNA IN NON-OLFACTORY TISSUES

Weiler E.[1] *[1]Neurophysiology, Ruhr-University Bochum, Bochum, Germany*

Odorant receptors were first cloned by Buck & Axel in 1991 from rat olfactory sensory neurons. An increasing number of odorant receptors were identified since then. Estimations approximate 1000 different olfactory receptor proteins, expressed specifically in olfactory neurons, responsible for odor detection. However, odorant-like receptors were also found in testis. Using RT-PCR we investigated systematically the expression of the mRNA of several odorant receptors in a variety of non-olfactory tissues (brain, cerebellum, eye, adrenal gland, kidney, heart, skeletal muscle, intestine, liver, testis, lung) and in the olfactory mucosa and olfactory bulb. Primers were designed to recognize specifically one type of odorant receptors (OR-F2, F3, F5, F6, F12, I3, I7, I8, I9, I14, I15; OR-nomenclature according to Buck & Axel, 1991). The expression pattern varied across tissues and odorant receptors: all odorant receptor mRNAs were found in olfactory epithelium and bulb, several mRNAs were found in liver, partially the same and others in kidney, testis, brain and the other non-olfactory tissues. None of the odorant receptor mRNAs were found in skeletal muscle. The function of odorant receptors in non-olfactory tissues is still unknown, however, other seven-transmembrane-domain receptors are known to function also as "chemo" receptors in chemical signal processing: as neurotransmitter and hormonal receptors. Dependent on the linkage to the signal cascade of G-proteins or other second messengers within the cell, the odorant receptors in non-olfactory tissues might act in a similar function or to detect metabolic substances.

242 Poster : Olfactory Receptor Genes

FUNCTIONAL ANALYSIS OF STABLY EXPRESSED MAMMALIAN OLFACTORY RECEPTORS

Bieri S.[1], Valero A.[1], Slack J.[2], Schilling B.[1] *[1]Givaudan Schweiz AG, Duebendorf, Zurich, Switzerland; [2]Givaudan Flavors Corp., Cincinnati, OH*

Recombinant expression of G protein-coupled receptors (GPCRs) in heterologous systems is widely used for high throughput screening of ligands. But to date most members of the olfactory receptor (OR) family, the largest family of GPCRs, are still orphan receptors. Heterologous expression of ORs is different to other GPCRs since the recombinant receptors are not readily transported to the cell surface. Consequently, the percentage of functional ORs that are accessible to odorants is low. In the past, several homologous and heterologous expression systems have been reported where ORs were characterised using low numbers of odour molecules. None of these systems was used in an automated screening procedure. In the present study mammalian ORs (such as OR17-40) were stably expressed in a heterologous system, resulting in a robust in vitro expression of the receptor. Ligand activation of the receptors was studied by monitoring fluxes of the internal calcium concentration in a microtiter plate format. Ligand specificities of the studied ORs were consistent with data in the literature and receptor activation by agonists occurred in a dose-dependent manner. Series of structurally related molecules were tested for their ability to activate the OR.

243 Poster : Olfactory Receptor Genes

THE ORPHAN RECEPTOR GUANYLYL CYCLASE EXPRESSED IN OLFACTORY SENSORY NEURONS OF RODENTS IS A PSEUDOGENE IN HUMAN: ASSIGNMENT TO CHROMOSOME BAND 11Q14

Nagatani G.[1], Wu K.[2], Fülle H.J.[2] [1]*PIBBS, University of Southern California, Los Angeles, CA;* [2]*Dept of Cell & Neurobiology, University of Southern California Keck School of Medicine, Los Angeles, CA*

Expression of the murine guanylyl cyclase-D (GC-D) gene (*Gucy2d*) is restricted to the dendritic cilia of a small population of olfactory sensory neurons that project to a group of atypical glomeruli in the olfactory bulb. These so-called 'necklace' glomeruli are thought to process olfactory cues associated with suckling behavior in neonatal rodents. Coexpression studies suggested that GC-D may function as a chemosensory receptor in a unique cGMP-signaling pathway (Fülle et al., 1995; Juilfs et al., 1997). Previously, we determined the chromosomal localization of *Gucy2d* by mouse interspecific backcross analysis. Close proximity of *Gucy2d* on mouse chromosome 7 to *Hbb* (hemoglobin beta-chain complex) and *Omp* (olfactory marker protein) suggested that the human ortholog gene maps to 11p15.4 or 11q13.4-q14.1 (Yang et al., 1996). Here, we used monochromosomal somatic cell hybrid and fluorescence in situ hybridization analysis to assign the human ortholog gene to chromosome 11q14. BLAST analysis of its genomic structure with mouse and rat cDNA sequences revealed multiple sequence differences causing open reading frame disruptions. This suggests that GC-D is a pseudogene in human that may not be transcribed or translated or encodes a degenerate, truncated protein similar to a large proportion of genes in the human heptahelical olfactory receptor repertoire.

244 Poster : Olfactory Receptor Genes

LOCALIZATION OF OLFACTORY RECEPTOR MRNA IN HUMAN OLFACTORY RECEPTOR NEURONS

Rawson N.[1], Yee K.[1], Hahn C.[2] [1]*Monell Chemical Senses Center, Philadelphia, Pennsylvania;* [2]*Dept. Psychiatry, University of Pennsylvania, Philadelphia, Pennsylvania*

Three approaches were used to establish the expression of olfactory receptor (OR) mRNA in human olfactory receptor neurons (ORNs). First, mRNA was extracted from biopsied olfactory epithelium and RT-PCR carried out with primers designed to amplify each of the chromosome 17 OR genes. Product was obtained only for 17-40, which was cloned and sequenced to confirm identity. In the second approach, OMP-immunoreactive ORNs were picked from sectioned olfactory tissue following on-slide reverse transcription using a t7-oligod(T) primer to enable reamplification (Eberwine et al., 2001). First and second round cDNAs were used as templates for PCR reactions with the same primer set. Only product for 17-7 was obtained with first round cDNA. Following reamplification, products were obtained for 17-7 and 17-40. Sequence identity of the cloned products was confirmed (ACC# X80391 and NM_014565). In the third approach, we localized mRNAs for 17-7 and 17-40 in primary cultures derived from human olfactory epithelium using in situ hybridization. Each probe labeled 1-2% of neurons in cultures from two different subjects. There was no labeling of non-neurons or co-expression in the same neuron, even when hybridized under very low stringency conditions. These data are the first to show that OR mRNA is, indeed, present in human ORNs. Expression in cultured human ORNs provides further evidence that these cultures are a valid system to study the cell and molecular basis of human olfaction. Funded in part by DC000214 (NR), DC004645 (KY), MH063946 and MH061912 (CH).

245 Poster : Olfactory CNS Coding

SPACE, TIME AND ODOR CODING I: ODOR IDENTITY WITHIN THE CONTEXT OF CONTEXT IN *MANDUCA SEXTA*

Cheich M.[1], Daly K.C.[1], Smith B.H.[1] [1]*Entomology, Ohio State University, Columbus, OH*

Odors are represented as spatiotemporal responses across populations of units in insect antennal lobes (ALs). These responses depend upon contextual parameters, such as repeated pulsing, pulse duration and inter-pulse interval (IPI). The current objective was to determine if odor identity (OID) could also be resolved in the spatiotemporal response of a population of units after accounting for stimulus context effects. Multichannel electrodes were implanted into the moth AL. Responses of up to 20 units were simultaneously recorded while presenting ten pulses of 4 alcohols and ketones at 5 different IPIs ranging from 500 ms to ~10 s. Each unit was sampled for 600 ms during each odor presentation; these data were then divided into sequential 30 ms bins. Factor analysis grouped units based on common response patterns across all stimuli. Analysis of factor score variance revealed significant IPI and OID effects. These effects were dependent on time, as evidenced by significant bin-by-IPI and bin-by-OID interactions. Importantly, OID main effects and interactions were hierarchically tested after all context effects and thus were significant under statistically stringent conditions. Furthermore, significant higher order interaction of bin-by-IPI-by-OID indicated that OID and IPI are interdependent as well as dependent on time. This suggests that spatiotemporal response patterns in the AL are the result of multiplexed, parallel processing of both odorant identity and stimulus context across the population of responsive units. Work supported by NIH-NCRR (R01 RR14166-06) to BHS & NIH-NIDCD (R03 DC05535-01) and McDonnell Foundation to KCD

246 Poster : Olfactory CNS Coding

SPACE, TIME AND ODOR CODING II: HIGH DIMENSIONAL EUCLIDIAN DISTANCE AS A MEASURE OF ODOR DISCRIMINABILITY AND TIME TO DISCRIMINATION ACROSS POPULATIONS OF ANTENNAL LOBE UNITS IN MANDUCA SEXTA.

Daly K.C.[1], Smith B.H.[1] [1]*Entomology, Ohio State University, Columbus, Ohio*

Behavioral studies suggest that discriminability of odors is related to the molecular features that make each odorant unique. Analysis of neural ensembles within the antennal lobe (AL) identified subsets of units defined by covariant temporal response patterns that were feature-dependent. The current analysis assesses how these subsets work collectively as a population to discriminate odorants over time and provides an indication of how long discrimination may take. Multiunit recordings from 2 moths were analyzed. Peri-stimulus responses were sampled for 780 ms from each unit in 30 ms bins. Principle Components Analysis extracted orthogonal factors whereby each represented common temporal response patterns of coactive units. General Linear Modeling identified factors with odor-dependent effects ($p < 0.001$). Odor-dependent factors were treated as an independent dimension, and the Euclidian distance between odors at each bin was calculated. The initial population response was a decrease in distance across odors at 90 ms post stimulus onset, which reflects an initial inhibition across the population in response to odor. In the next 90-120 ms the distance among odors was maximal, suggesting that optimum discriminability may occur at this time epoch. Finally, we show that the population response distance was dependent on the absolute difference between odorant features; this is consistent with our prior behavioral data. Work supported by NIH-NCRR (9 R01 RR14166-06) to BHS & NIH-NIDCD (1 R03 DC05535-01) and McDonnell Foundation grants to KCD

247 **Poster : Olfactory CNS Coding**

ODOR RESPONSES OF PROJECTION NEURONS INNERVATING MORPHOLOGICALLY IDENTIFIED GLOMERULI IN THE ANTENNAL LOBE OF THE MOTH *MANDUCA SEXTA*

Reisenman C.[1], Christensen T.A.[1], Hildebrand J.G.[1] [1]*ARL Neurobiology, University of Arizona, Tucson, AZ*

The antennal lobe (AL) of the moth *M. sexta*, like the vertebrate olfactory bulb, contains an array of glomeruli, sites of initial processing of olfactory information. The moth's AL also contains sexually dimorphic glomeruli: the macroglomerular complex (MGC) in males and the large female glomeruli (LFGs) in females. The role of the MGC as the site of pheromone information processing is well established, but little is known about the functional properties of the sexually isomorphic glomeruli. We used intracellular recording and staining to examine the odor responses of 16 uniglomerular projection (output) neurons (PNs) innervating several of these glomeruli. Five PNs with arborizations restricted to the same glomerulus show strong, selective excitatory responses to nano-to-picomolar concentrations of *cis*-3-hexenyl-acetate, a key plant volatile. Structurally related odorants, such as *cis*-3-hexenyl-propionate and 2-octanone also evoked excitatory responses, but at concentrations 1-3 log steps greater. In several cases, these PNs showed inhibitory responses to (±)-linalool, an odorant that selectively excites PNs innervating the lateral LFG, a neighboring glomerulus. Thus, the excitatory response to *cis*-3-hexenyl-acetate may be modulated by local inhibitory interactions. These results provide evidence that uniglomerular PNs innervating two neighboring glomeruli are narrowly tuned to host-plant volatiles that belong to different chemical classes. This suggests that, as shown for the MGC in males, glomeruli that are not specialized for pheromone information processing are also organized chemotopically. Supported by a PEW fellowship to CR and NIH grant R01-DC-02751 to JGH

248 **Poster : Olfactory CNS Coding**

PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF CO_2-SENSITIVE NEURONS IN THE ANTENNAL LOBE OF THE MOTH *MANDUCA SEXTA*

Guerenstein P.G.[1], Hildebrand J.G.[1] [1]*ARL Division Of Neurobiology, University of Arizona, Tucson, Arizona*

We are investigating how the central nervous system of the moth *Manduca sexta* processes environmental-CO_2 information from specialized receptor cells in the labial palp organ (LPO) in the mouthparts. *M. sexta* possesses a highly developed CO_2-detecting system, and we have already shown that the antennal lobe (AL) is the site of first-order processing of CO_2 information in the moth's brain. Moreover, we have reported on AL projection neurons (PNs) that responded to CO_2 stimuli and arborized in a specific glomerulus in the AL, the LPO glomerulus (LPOG). By using intracellular recording and staining techniques, we have obtained new results that suggest that different types of neurons in or associated with the AL process CO_2 information. These include PNs, as already described, local interneurons, and at least one type of bilateral neuron with the cell body in the protocerebrum (PC). The latter neuron arborizes in the LPOG of both ALs and sends a neurite to the lateral horn of the PC of one hemisphere. We also have evidence that some AL CO_2-sensitive neurons integrate CO_2 information from the LPOs with antennal inputs, while other CO_2-sensitive neurons do not respond to the input from the antenna. While it is known that CO_2 information is used by many insect species in vital tasks such as locating food sources, its role in the moth's biology is not well understood. We hope that by complementing our findings with those from ongoing projects on behavioral responses of the moths to CO_2 stimuli, we will be able to reveal roles of CO_2 for these insects. Supported by NSF grant IBN-0213032.

249 **Poster : Olfactory CNS Coding**

GENETIC CONTROL OF OLFACTORY CHARACTERISTICS IN MALE MOTHS.

Vickers N.J.[1] [1]*Biology, University of Utah, Salt Lake City, Utah*

Recent studies have suggested that novel female moth pheromone blends might arise through `saltational shifts´ but relatively little is known of the mechanisms by which male olfactory systems might `track´ such changes to produce narrowly tuned behavioral responses. Studies of the pheromone-mediated behaviors and underlying peripheral and central olfactory neurophysiology in two closely related heliothine moth species have revealed the types of changes that have occurred in the olfactory system as these two species diverged. One of the main changes that has accompanied the divergence of *Heliothis virescens* and *Heliothis subflexa* has been the switch from Z9-14:Ald to Z9-16:Ald as the essential secondary component in the pheromone mixture. Because these two species can hybridize under laboratory conditions, studies can also be made of the genetic control of olfactory characteristics. Hybrid males were unable to discriminate between the presence of these compounds, responding equally to blends containing either Z9-14:Ald or Z9-16:Ald. Backcross males responded as either parental type (depending upon the direction of the backcross) or hybrid-like males in a 1:1 ratio. This suggests that a single autosomal allele controls the response phenotype. The behavioral preference phenotypes displayed by hybrid and backcross male moths were correlated with the specificity of central interneurons arborizing within a specific olfactory glomerulus of the male macroglomerular complex. Thus, changes in the olfactory system possibly controlled by single alleles might account for the ability of males to respond to novel pheromone blends. Supported by NSF IBN-9905683.

250 **Poster : Olfactory CNS Coding**

WHOLE-CELL PATCH-CLAMP RECORDINGS FROM KENYON CELLS IN AN IN VIVO PREPARATION OF THE MOTH BRAIN

Sjöholm M.[1], Hansson B.[1] [1]*Swedish University of Agricultural Sciences, Alnarp, Sweden*

Previous studies on insect neurophysiology have employed intracellular techniques with sharp electrodes or whole-cell patch-clamping on dissociated cells to record and stain individual neurons. Both methods have drawbacks, however. Sharp electrodes mainly impale larger neurons and it´s impossible to aim for a specific cell. On the other hand, whole-cell recordings from dissociated cells do not provide any information about the physiological context of the neurons.

Here, we apply whole-cell patch-clamping to an *in vivo*preparation of the intact moth brain in order to record and stain Kenyon cells, which are the very thin neurons that comprise the mushroom bodies. The mushroom bodies are paired neruopils which receive input from several sensory modalities and have proven to be important for olfactory learning . Kenyon cell somata were recorded in whole cell mode under both voltage clamp and current clamp conditions. Both electrical stimulation via the recording electrode and olfactory stimuli delivered to the antennae were empolyed. All the recorded cells expressed voltage-activated currents upon depolarization. Most of them could sustain action potentials elicited by depolarizing current pulses in current clamp configuration. A few responded to olfactory stimuli delivered to the antennae with depolarization and spikes. The cells were readily stained and their morphology was clear even at great detail.

This method may be a powerful tool in combination with e.g. multi unit electrodes to futher elucidate coding and processing of olfactory signals in the insect brain.

251 Poster : Olfactory CNS Coding

ENSEMBLE CODES FOR CONTEXTUAL FEATURES OF OLFACTORY STIMULI RECORDED WITH MULTI-ELECTRODE ARRAYS IN THE MOTH ANTENNAL LOBE

Dacks A.[1], Christensen T.A.[1], Pawlowski V.M.[1], Hildebrand J.G.[1] [1]*ARL Division of Neurobiology, University of Arizona, Tucson, AZ*

For many animals, the task of identifying and locating the source of an odor depends on the ability to track the stimulus even as its physical features change in space and time. Moths like *Manduca sexta* are well adapted behaviorally to track odor plumes that are both dynamic and unpredictable. We therefore examined the effects of varying certain stimulus parameters such as pulse duration and concentration on the neural representations of plant odors at the first stage of processing in the antennal lobe. Neural-ensemble recording using a 4-pronged silicon multielectrode array was used to record the odor-evoked population activity across several glomeruli. We found that irrespective of stimulus duration, there is only a brief window of time at the beginning of the response during which the ensemble pattern remains consistent from trial to trial. Outside this window, responses to consecutive pulses show much more variation. When temporal correlations between units were examined, we found that odor-evoked synchronized firing was not periodic, but was locked to the same brief time window. As found previously in the pheromone subsystem, repeated stimulation with plant odors also led to a decrease in correlated firing over time. Finally, elevated stimulus concentrations triggered an increase in ensemble activity, and the resulting ensemble representations evoked by different odorants became less discriminable. These results show that odor representations do not only encode odor identity, but also carry information about contextual features of the stimulus that change unpredictably with time. Supported by NSERC fellowship PGSA-244345-2001 to AD and NIH grant R01-DC-02751 to JGH.

252 Poster : Olfactory CNS Coding

MALE AND FEMALE FERRET ANAL SCENT GLAND SECRETIONS ACTIVATE DIFFERENT CLUSTERS OF GLOMERULI IN THE MAIN OLFACTORY BULB.

Woodley S.K.[1], Sapute S.[1], Lieberman Z.[1], Coulter-Thurley R.[1], Baum M.[1] [1]*Biology, Boston University, Boston, MA*

In ferrets, body odors detected by the main olfactory system are required to identify opposite-sex mating partners. Volatile anal scent gland secretions, which vary quantitatively in male and female ferrets, may be one source of the odors contributing to mate recognition. We hypothesized that anal scents derived from the 2 sexes would activate distinguishable populations of glomeruli in the main olfactory bulb (MOB) of female ferrets and that estradiol may modify this profile of activation. Estrous female ferrets were exposed to volatile anal scents diluted 1:200 or fresh air for 30 minutes and then killed 60 minutes later. Olfactory bulb sections were processed for Fos-IR, a marker of neuronal activity. Activated glomeruli, identified by the presence of Fos-IR in juxtaglomerular cells, were spatially mapped using a computer algorithm (Schaefer et al., 2001). Male and female anal scents activated overlapping but distinguishable clusters of glomeruli in the caudal-ventral MOB. Activation induced by male anal scents was very similar to that seen in other estrous females after they had mated with a male. There was no difference in MOB glomerular activation evoked by male anal scents in ovariectomized females treated with estradiol or oil vehicle. Differential activation of clusters of MOB glomeruli by male versus female anal scents may be the first step in gender discrimination. (Supported by NIH grant HD21094 and Fellowship DC 00426-03) Reference: Schaefer, ML, Young DA, and Restrepo D. 2001. J. Neuroscience, 21:2481-87.

253 Poster : Olfactory CNS Coding

CHANGES IN ODOR-EVOKED C-FOS EXPRESSION PATTERNS ELICITED BY OPERANT CONDITIONING IN MOUSE OLFACTORY BULB

Zhang C.[1], Arellano J.[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, Colorado*

Mice can readily learn to detect odors in an operand conditioning task (Bodyak and Slotnick 1999). In this study, we investigate whether exposure of adult mice to this learning task induces long term changes in odor-evoked *c-fos* gene expression in the olfactory bulb. One group of FVB mice (TEA) was water deprived, introduced to the operand conditioning chamber, and underwent extensive operant conditioning training for odor detection for ethyl acetate while another group (UEA) underwent water deprivation and was introduced to the chamber, but did not undergo training. Once the threshold for ethyl acetate was determined for the TEA group, both groups of mice had received free access to water for a week. Mice were then exposed to ethyl acetate for 30 min in a 5 l glass jar. We measured *c-fos* mRNA expression in periglomerular cells by *in situ* hybridization and utilized a mapping method developed in our laboratory (Schaefer et al. 2001) to determine regional activation of glomeruli in the olfactory bulb. We find not only that more glomeruli were activated in TEA mice but also that the olfactory bulb of TEA mice exhibit odor-evoked *c-fos* activity patterns substantially different from that of UEA. Our study indicates that long term changes in modulation of *c-fos* gene expression take place after learning in this operand conditioning task underscoring the high capability for neural plasticity and learning in the olfactory bulb. This work was supported by grants DC00566, DC00244 and DC04657 from the NIDCD.

254 Poster : Olfactory CNS Coding

A FOVEA IN THE NOSE: EVIDENCE FOR DISPROPORTIONATE MAPPING OF THE OLFACTORY NOSE ONTO THE MAIN OLFACTORY BULB OF THE HAMSTER

Schoenfeld T.A.[1], Knott T.K.[1] [1]*Physiology, University of Massachusetts Medical School (Worcester), Worcester, Massachusetts*

Olfactory receptor neurons (ORNs) in the nose project to the main olfactory bulb (MOB) of rodents in mutually exclusive streams that connect rhinotopically distinct nasal air channels and zones with the brain. To ascertain whether such topography entails the disproportionate representation seen in other sensory maps, stereological techniques were used to estimate the population sizes of ORNs in the olfactory epithelium (OE) of hamsters and their targets in the MOB (glomeruli, mitral and tufted cells). Each circumferential half of the MOB was found to contain about 50% of the 3000 glomeruli and about 50% of the 160,000 mitral and tufted cells per bulb. Equivalent numbers of mature ORNs were found to project from the medial and lateral recesses to the medial and lateral halves of the MOB, respectively (4.5 million each). However, far fewer mature ORNs project from central channel OE to the dorsal MOB (2 million) than from peripheral channel OE to the ventral MOB (7 million). Thus, there is a disproportionate mapping of the central-peripheral OE axis onto the MOB, encompassing a 3-fold-plus variation in the convergence of ORNs onto MOB secondary neurons. Inspired air reportedly flows along the central-peripheral axis, and spatial variation in the expression of odorant receptor genes is largely restricted to this axis. Controlled sniffing, therefore, may serve to direct odorants to particular central-peripheral zones, to stimulate optimally-tuned ORNs that converge onto MOB neurons with an optimal balance of resolution and sensitivity. By this anatomical design, sniffing may operate somewhat like foveation in vision. Supported by NIH grant DC03835.

255 Poster : Olfactory CNS Coding

EXPERIENCE-INDUCED OLFACTORY BULB MITRAL/TUFTED CELL RECEPTIVE FIELD PLASTICITY

Fletcher M.L.[1], Wilson D.A.[2] [1]*Zoology, University of Oklahoma, Norman, Oklahoma;* [2]*Department of Zoology, University of Oklahoma, Norman, Oklahoma*

In the olfactory system, experience and learning have been shown to modify neural response patterns as well as enhance behavioral olfactory acuity. In spite of these known experience-induced changes, it is unclear whether this plasticity translates into changes in olfactory bulb odor coding. One way in which this plasticity could be examined is through quantitative analyses of olfactory system receptive fields (RFs). The following experiment aimed at better understanding olfactory bulb mitral/tufted cell receptive fields, the role of experience in olfactory system odor coding, and receptive field plasticity. Single-unit recordings were made from mitral/tufted cells of freely breathing urethane-anesthetized rats. RFs to ethyl esters ranging from ethyl formate to ethyl octanoate were mapped followed by adaptation to one odor within the receptive field. Preliminary results revealed that the best odorant for each cell varied within the odorant series, with a mean statistically significant response to 3 odorants in the series (range:0-5 odorants). RFs were then remapped in the adapted and recovered state (up to 1 hour). Results are consistent with a feature detection model of mitral/tufted cell function in which adaptation to one odorant within the RF partially suppresses the entire RF. It is also shown that experience can alter the overall shape of the RF by shifting the RF toward the experienced odorant. Supported by: DC03906 to DAW

256 Poster : Olfactory CNS Coding

REPRODUCIBILITY (OR LACK THEREOF) IN GLOMERULAR ACTIVATIONS

Kida I.[1], Xu F.[1], Hyder F.[1] [1]*Diagnostic Radiology, Yale University, New Haven, CT*

Determining the reproducibility of odorant-induced glomerular responses is important for reliable interpretation of results from olfactory bulb (OB) experiments. Here we discuss assessment of single-subject test-retest (TrT) reliability from fMRI studies in the entire OB. Results from mice and rats are used to demonstrate that TrT reliability is dependent on spatial resolution and contrast to noise ratio. While visual comparison of repeated activity patterns throughout the entire OB can be informative, TrT reliability can be meaningfully defined and quantified per glomerular cluster. The 2DG autoradiography method applied to image the entire OB revealed a number of basic principles in organization of glomerular maps, but confirming reproducibility of activity patterns has been qualitative because TrT reliability cannot be performed with 2DG. The c-fos staining method for imaging the entire OB poses similar challenges and limitations. Although other methods – electrophysiologic recordings, voltage sensitive dyes, Ca2+ imaging,„ intrinsic imaging – can provide TrT reliability, these methods suffer from restricted field of view of detection and often TrT reliability cannot be assessed because of signal averaging. While neuroanatomical studies suggest that glomeruli are similar to other synaptic structures in the CNS – columns or barrels in the cortex – the physiological response to an odorant is activation of large clusters of glomeruli located "unconnectedly" throughout the entire OB. Thus fMRI is ideally suited to quantitatively assess TrT reliability. Supported by NIH (NS-037203, DC-003710, MH-067528) and NSF (DBI-0095173) grants.

257 Poster : Olfactory CNS Coding

SPATIAL PATTERNS OF ODOR-EVOKED ACTIVITY IN PIRIFORM CORTEX CHANGE DURING DEVELOPMENT

Illig K.R.[1] [1]*Psychology, University of Virginia, Charlottesville, VA*

Presentation of certain monomolecular odors evokes activity in spatially restricted patches of cells in the rodent olfactory bulb (OB). In piriform cortex (PC), odor-evoked activity is found in rostrocaudally-oriented bands of cells in anterior PC (APC), likely indicating distinct subregions, while activity is found throughout posterior PC (PPC; Illig & Haberly, 2003). To determine whether these patterns are present at birth and whether they are modified during early postnatal life, odor-evoked activity was assessed in developing rats (P3-P30). Animals were exposed to single aliphatic acid odorants in odor-free chambers, and anaesthetized and perfused 60 minutes later. Odor-evoked activity was visualized by detection of Fos protein. In OB, Fos-like labeling was present in the granule cell, mitral cell and glomerular layers at the earliest ages examined, with staining clustered in areas previously reported as active in response to these odors (Guthrie & Gall, 2003). In PC, activation in layers II/III shared some features with that seen in the adult: in APC, rostro-caudally oriented bands of Fos-positive cells alternated with bands relatively free of label, and labeled cells were found throughout PPC. However, in P3-P7 animals, the pattern of activation in APC was nearly opposite to the adult pattern; pups exhibited a central band of activated cells that was flanked by two bands relatively free of Fos-positive cells. The adult pattern (a central cell-poor band flanked by cell-rich bands) emerged by P10. These results suggest that subregions of APC are functionally distinct shortly after birth, and that the roles of these subdivisions in odor processing may shift during the second postnatal week. Supported by DC0038.

258 Poster : Gustatory Transduction

CALCIUM PUMP ISOFORMS IN CHEMOSENSORY TRANSDUCTION

Gannon-Murakami L.[1], Yano J.[1], Valentine M.[1], Preston R.R.[2], Zhukovskya M.[2], Van Houten J.L.[1] [1]*Biology, University of Vermont, Burlington, VT;* [2]*Pharmacology, Drexel University, Philadelphia, PA*

Paramecia are attracted to stimuli through 3 pathways, at least two of which involve the plasma membrane calcium pump (PMCA) to sustain attractant-induced hyperpolarization. Paramecia express at least 3 PMCA isoforms; over-expression of two isoforms leads to both defective pump activity and chemoresponse, presenting an opportunity to probe for the role of PMCA activity in chemoresponse. 1) GFP-isoform-2 or HA-isoform-3 transformants show correlations between defective pump activity and chemoresponse: GFP-isoform 2 leads to defects specifically in folate chemoresponse; HA-isoform 3 leads to general decline in chemoresponse with the most profound defect in folate chemoresponse 2) Over-expression of isoforms without tags also leads to defects in Ca extrusion and in chemoresponse; the defects in chemoresponse are specific to each PMCA isoform and in agreement with results with the tagged PMCAs. 3) Voltage clamp studies show reduced Ca conductance in the the HA-isoform 3 transformants, consistent with elevated intracellular calcium. 4) Voltage clamp shows outward conductance of a 120-250 pA upon application of uM glutamate, and slight inward current upon removal, with studies of PMCA transformants underway. 5) Over-expression of PMCA regulatory domains regions (CBDs): HA-CBD isoform 2 transformant shows defective calcium extrusion, and variable effects on chemoresponse that are being studied further.Support by DC 00721, DC 05308, GM59988 and NCI PHS 22435.

259 Poster : Gustatory Transduction

GPI ANCHORED PROTEINS AND LIPID RAFTS IN CHEMOSENSORY SIGNAL TRANSDUCTION

Yano J.[1], Valentine M.[1], Chandran S.[1], Weeraratrane S.[1], Kasper S.[1], Pan Y.[1], Preston R.R.[2], Van Houten J.[1] *[1]Biology, University of Vermont, Burlington, Vermont; [2]Pharmacology and Physiology, Drexel University, Philadelphia, PA*

Glycosyl phosphatidylinositol (GPI) anchored proteins attach to the outer leaflet of the plasma membrane through a lipid anchor and congregate in lipid rafts, that are domains enriched in sphingolipids and cholestero. Rafts serve to organize signaling molecules in other systems, and also surface molecules like the calcium pumps (PCMAs). In Paramecium, signal transduction for at least two of three pathways appears to require activation of PCMAs to sustain the stimulus induced hyperpolarization that leads to attraction, and at least one stimulus has a GPI anchored protein as its receptor. We show here with confocal microscopy that a GPI anchored chemoreceptor for folate localizes with the PCMAs and another GPI anchored protein at the bases of cilia. Sucrose gradient analysis shows that GPI anchored proteins associate with low density lipid fractions, i.e. lipid rafts. Solubility and immunoprecipitation studies show that PMCA isoform 2 is relatively insoluble in detergent lipid rafts. Also, the PCMAs are in larger complexes where they can be crosslinked. In contrast, a transmembrane chemoreceptor does not localize with the PMCAs or GPI anchored proteins. Our model of signal transduction is that GPI anchored chemoreceptors cluster in lipid rafts at the bases of cilia where they associate with a transmembrane protein and signal to channels and PCMAs to hyperpolarize and sustain the hyperpolarization of the cells in attractant stimulus. Supported by GM59988, DC 00721, NCI PHS 22435.

260 Poster : Gustatory Transduction

GUSTATORY GENE EXPRESSION PROFILING

Nosrat I.[1], Farjo R.[2], Othman M.[2], Swaroop A.[2], Nosrat C.A.[1] *[1]Laboratory of Oral Neurobiology, Biologic & Materials Sciences, University of Michigan, Ann Arbor, Michigan; [2]Department of Ophtalmology, University of Michigan, Ann Arbor, Michigan*

While there is an abundance of information regarding the development of many sensory organs from a mole-cular point of view, little information is available about the development and maintenance of the gustatory system. Only few genes have been characterized in the taste system and this has been generally based on studies that have characterized a single or a limited number of gene(s). In order to gain further understanding about the molecular aspects of the taste system, we have used microarray technology to characterize the gustatory gene expression profiles. Microarrays constitute an emerging and rather new technology with tremendous promise, providing the tools necessary for investigating the expression and interaction of thousands of genes simultaneously. Taste buds develop from the lingual epithelium, and therefore, a comparative gene analysis of the lingual and the gustatory epithelia would provide us with a differential gene expression profile for the peripheral taste system. In contrast to common belief, our initial analysis of gene expression profiles shows that many genes are less abundant in the gustatory epithelium compared to the lingual epithelium. This indicates that down-regulating the expression of epithelial genes, in combination with turning on "taste-specific" genes, would be the determinant for the gustatory phenotype of the lingual epithelium. We aim to characterize such regulatory genes.

While much remains to be done, characterizing the gustatory gene profile will facilitate the elucidation of pathways that constitute complex biological processes of induction, development, maintenance and disease pathogenesis.

261 Poster : Gustatory Transduction

EXPRESSION PROFILING OF TASTE RECEPTOR CELLS.

Lopez-Jimenez N.D.[1], Sainz E.[1], Blackwood C.A.[1], Cavenagh M.M.[1], Battey J.F.[1], Sullivan S.L.[1] *[1]National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Rockville, MD*

In order to molecularly characterize taste receptor cells (TRCs), we are expression profiling TRCs using two methods. The first is the sample sequencing of a normalized, subtracted TRC-enriched cDNA library. To date, 20,000 clones from the library have been sequenced. As evident from the abundance of known TRC-specific genes, including members of both the Tas1R and Tas2R families of taste receptors, the library is highly enriched in genes expressed by TRCs. Among the library clones, we identified and are further characterizing several novel ESTs which are selectively expressed in TRCs. As a second approach to identifying TRC-expressed genes, massively parallel signature sequencing (MSPP) of cDNAs generated from mRNA isolated from taste buds is being performed. MSPP analysis is a microbead based technique that permits the signature sequencing of millions of cDNAs derived from any given mRNA population. Since MSPP analysis is both representative and comprehensive, we expect this approach will allow us to identify rare mRNAs expressed by TRCs as well as determine the relative abundance of different mRNA species. This work is supported by the Division of Intramural Research, NIDCD/NIH.

262 Poster : Gustatory Transduction

EFFECT OF Ni^{2+} ON VOLTAGE-DEPENDENT Na^+ CURRENT IN MORPHOLOGICALLY IDENTIFIED CELLS OF THE BULLFROG TASTE ORGAN

Suwabe T.[1], Narita K.[2], Okuda-Akabane K.[2], Kubota M.[1], Kitada Y.[2] *[1]Department of Operative Dentistry and Endodontics, School of Dentistry, Iwate Medical University, Morioka, Japan; [2]Department of Oral Physiology, School of Dentistry, Iwate Medical University, Morioka, Japan*

We have reported that the responses of the frog glossopharyngeal nerve to NaCl are enhanced by addition of $NiCl_2$ to NaCl stimulating solution. This suggests that taste cells sensitive to $NiCl_2$ exist in the frog taste organ. The frog taste organ contains three types of cells: type Ib, type II and type III cells. These cells are electrically excitable because they contain voltage-gated Na^+ and K^+ channels. In this study, we examined the effects of $NiCl_2$ on the voltage-dependent inward sodium currents (I_{Na}) of the three types of morphologically identified taste cells of the bullfrog (*Rana catesbeiana*) taste organ by whole-cell patch clamp recording with a Lucifer yellow-filled pipette. Slice preparations of taste organs were used. Ringer's solution containing 1 mM $NiCl_2$ prolonged the inactivation process of I_{Na} of three types of cells to different degrees. The time course of inactivation of I_{Na} was fitted with a single exponential function. The decay constants of I_{Na} in type Ib, type II and type III cells were 1.0 ± 0.1 ms (mean ± SEM; $n = 16$), 0.7 ± 0.1 ms (16) and 4.3 ± 0.3 ms (17) without Ni^{2+} and 2.6 ± 0.3 ms, 3.4 ± 0.8 ms and 24.3 ± 2.4 ms with 1 mM Ni^{2+}, respectively. These results indicate that Ni^{2+} has a much stronger effect on type III cells than on type Ib and type II cells. The action of Ni^{2+} on type III cells may be responsible for enhancement of the neural response to NaCl by Ni^{2+}.

263 Poster : Gustatory Transduction

RAT TASTE BUDS EXPRESS MULTIPLE MEMBERS OF THE KCNK FAMILY OF TWO-PORE DOMAIN POTASSIUM CHANNELS.

Burks C.A.[1], Hansen D.R.[1], Rao S.[1], Lin W.[2], Kinnamon S.C.[3], Gilbertson T.A.[1] *[1]Biology, Utah State University, Logan, UT; [2]Cellular & Structural Biology, University of Colorado Health Sciences Center, Denver, CO; [3]Anatomy & Neurobiology, Colorado State University, Fort Collins, CO*

Two-pore domain K (K_{2P}) channels form K^+-selective pores in cells that allow passive K^+ transport that is important for K^+ homeostasis, cell volume regulation and the control of electrical excitability. Individual K_{2P} channels respond to such stimuli as acidic pH and fatty acids making them potentially important as transduction channels in the taste system. Using RT-PCR we have probed mRNA from taste buds of the fungiform, foliate and vallate papillae in rat tongue. Primers were designed for various K_{2P} channels, which are part of the KCNK gene family, including TWIK (Tandem of P domains in Weak Inward rectifier K^+ channels), TASK (TWIK-related Acid-Sensitive K^+ channels), TREK (TWIK-related K^+ channels) and TRAAK (TWIK-related Arachidonic Acid-activated K^+ channels). Similar to other tissues that express a wide variety of K_{2P} channels, each of the three taste bud types expresses several members of this family. To date, we have found TWIK1, TASK1-3, TREK1-2, and TRAAK1 in foliate and vallate taste buds; of these, fungiform taste buds lack TASK3, TREK2 and have low TASK1 expression. Sequencing of the PCR products has confirmed their identity. Currently, we are attempting to quantify expression of these K_{2P} channels using a multiplexed TaqMan-style real time PCR assay. The function of K_{2P} remains unclear but they remain attractive candidates for contributing to the transduction of acids and fatty acids in addition to their roles in mediating cell homeostasis. Supported by NIDDK 59611 & UAES 00630 (TAG).

264 Poster : Gustatory Transduction

FUNCTIONAL IMPLICATIONS OF DIFFERENCES IN POTASSIUM CHANNEL EXPRESSION AMONG LINGUAL TASTE BUDS.

Hansen D.R.[1], Hoyal D.O.[1], Foley C.E.[1], Guenter J.[1], Johnson D.J.[1], Gilbertson T.A.[1] *[1]Biology, Utah State University, Logan, UT*

Delayed rectifying K^+ (DRK) channels in mammalian taste receptor cells (TRCs) are important for repolarization of the cell following activation and may serve as direct and indirect targets for modulation by taste stimuli. Using a multiplexed TaqMan-style assay in a real-time quantitative PCR system, we show that there are significant differences in expression of the nine major types of DRK channels among taste buds of the fungiform (Fu), foliate (Fo) and vallate (Va) papillae of the rat tongue. To determine if there are functional consequences to these differences in DRK channel expression, we have used patch clamp recording to characterize the biophysical properties of outward potassium currents in isolated taste buds from these three papillae. On average, the properties of current activation, inactivation, deactivation and use-dependent inactivation were not significantly different among the taste bud types. However, a small subset of Fo and Va TRCs showed a different current profile suggesting that there might be significant cell-to-cell variability. To determine if there are functional differences in chemosensitivity among the taste buds, we looked at the affinity and specificity of fatty acids, which are known to inhibit DRK channels in Fu TRCs. Fu TRCs respond to only the *cis*-polyunsaturated fatty acids (PUFA) with an EC_{50} of ~1 μM. While Fo and Va TRCs respond to PUFAs, they also respond to a subset of monounsaturated fatty acids. Thus, differences in DRK expression may be accounting for the different specificity to fatty acids in the posterior TRCs. Supported by NIDDK 59611 (TAG).

265 Poster : Gustatory Transduction

MOUSE TASTE CELL ACTIVITIES IN RESPONSE TO TASTE STIMULI

Yoshida R.[1], Ishizuka S.[2], Lindemann B.[3], Ninomiya Y.[1] *[1]Oral Neurosicence, Kyushu University, Fukuoka, Japan; [2]Brain Science and Engineering, Kyushu Institute of Technology, Kitakyushu, Japan; [3]Physiology, Saar University, Homberg, Germany*

Responses of single receptor cells to taste stimuli have been studied by various methods including an in vivo intracellular recording and a patch clamp recording from isolated taste bud cells. There are, however, still many difficulties and problems on stable recording of single cell responses to reliable taste stimulation. We newly developed an experimental setup which allows application of taste stimuli to only apical side of the receptor membrane and recording responses from the basolateral side of the taste cell. In this setup, single taste bud of fungiform papilla was excised from peeled epithelium and held the mucosal surface in the orifice of the stimulating pipette. Electrical signals were recorded by using a loose patch configuration. Under this condition, we recorded action currents from a few percent of a total of taste cells tested for taste stimulation (NaCl, Saccharin, HCl, Quinine HCl). Our preliminary data indicate that many taste cells responded to one of the four taste stimuli and the others responded to multiple stimuli. There exist two groups of NaCl-responsive cells, one showing amiloride inhibition of NaCl response but the other not. Most of our results were comparable with data previously obtained from different experimental methods. Thus, our newly developed experimental setup may provide a powerful tool for recording of reliable single cell activities in response to taste stimuli exclusively applied to the apical membrane.

266 Poster : Gustatory Transduction

G PROTEINS AND ADENYLYL CYCLASES CO-EXPRESSED IN TASTE RECEPTOR CELLS

Abaffy T.[1], Trubey K.[1], Chaudhari N.[1] *[1]Physiology and Biophysics, University of Miami, Miami, FL*

The second messenger, cAMP, is implicated in transduction of sweet, umami and bitter taste qualities. For instance, sucrose increases, while glutamate and denatonium decrease cAMP levels. Membrane-bound adenylyl cyclases (AC) are the principal source of receptor-triggered synthesis of intracellular cAMP. We have previously detected the presence of AC 4, 6 and 8 in rat taste cells, both at mRNA and at the protein level. Known functional characteristics of ACs suggest that each could be involved in transduction pathway of one or more taste qualities. Prominent Galpha subunits in taste cells are Galphai2 and Galpha gustducin. First, we validated commercial antibodies using immunoblots. We used double-label fluorescent immunohistochemistry to examine which Galpha subunits are present in the cells that express each AC isoform. AC4 and AC6 are each found in 5-6 cells per taste bud profile. None of the AC4 positive cells stained for either gustuducin or Galphai2. Only a few cells that stained weakly for AC6 also showed immunoreactivity for both Gai2 and gustducin. On the other hand, AC8 immunoreactivity was frequently found in cells that express gustducin. 200 AC8 positive taste receptor cells from rat circumvallate papilla were examined. We found that 66%±19% of the cells positive for AC8, were also gustducin positive. Conversely, 57%±8% of the gustducin positive cells also expressed AC8. Since circumvallate papilla have only 10-20% cells gustucin positive, the overlap appears to be at a probability higher then chance. Because AC8 can be activated by Galphas and Ca, and inhibited by certain Gbetagamma subunits, the coexpression pattern may have implications for taste signal transduction. Supported by NIH (DC03013).

267 Poster : Gustatory Transduction

COMPARISON OF THE RESPONSES TO DIFFERENT SWEET AND BITTER STIMULI IN □-GUSTDUCIN KNOCKOUT (KO) AND WILD TYPE MICE (WT)

Danilova V.[1], Damak S.[2], Glendinning J.[3], Margolskee R.F.[2], Hellekant G.[1] *[1]University of Wisconsin, Madison, WI; [2]Mount Sinai School of Medicine, New York, NY; [3]Barnard College, Columbia University, New York, NY*

Because the role of α-gustducin in taste responses has been determined with only two bitter and sweet stimuli we decided to study the taste responses in KO and WT mice to an extensive series of sweet and bitter compounds. Whole chorda tympani (CT) and glossopharyngeal nerve (NG) responses to 11 sweeteners and 16 bitter compounds were obtained in KO and WT mice. Two bottle preference (TBP) and lickometer tests were also carried out. Generally, the responses to sweeteners were diminished in KO mice. However, they still showed a residual CT and/or NG response and preference at higher sweetener concentrations, except for NC00174 and D-tryptophan, which did not elicit responses at any concentration. In regard to bitter stimuli, the NG, not CT, nerve responses to the denatonium benzoate, cycloheximide, PROP, TEA, atropine, amiloride, QHCl, brucine, quinacrine chloroquine, sparteine and MgSO4, were diminished in KO mice, whereas responses to strychnine, urea, L-phenylalanine and caffeine did not differ between two groups. In TBP tests KO mice rejected denatonium, quinacrine and brucine at higher concentrations than WT. This study shows that α-gustducin is involved in transducing responses to most sweet and bitter compounds, although approximately one fourth of the bitter compounds tested apparently did not involve α-gustducin in their transduction. Supported by NIH DC03155 (R.M) and NIH DC005336 (V.D.)

268 Poster : Gustatory Transduction

CAP AND COFILIN-1 CO-EXIST IN TASTE CELLS EXPRESSING TASTE RECEPTORS

Ishimaru Y.[1], Emori Y.[2], Abe K.[1] *[1]Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan; [2]Department of Biophysics and Biochemistry, Faculty of Science, The University of Tokyo, Tokyo, Japan*

We have identified an actin regulatory factor, CAP, which is expressed in a subset of rat taste bud cells.[1)] Recently, another actin regulator, cofilin, was found to be associated with CAP, as reported by Moriyama *et al.*[2)], who suggested that CAP functions as a regulator of cofilin as well as G actin-binding protein by biochemical analyses. We then investigated the existence of cofilin in rat taste buds and found that one of cofilin isomers, cofilin-1, is co-expressed with CAP. In taste buds, the cells expressing CAP and cofilin-1 are identified as mature taste cells expressing the calcium-signaling cascade components such as phopholipase C-β2 and inositol trisphosphate receptor type 3 and two families of taste receptors.[3)] These results suggest that CAP and cofilin-1 are coordinately involved in the regulation of actin cytoskeleton in mature taste cells.

1) Y. Ishimaru *et al.* (2001) An actin-binding protein, CAP, is expressed in a subset of rat taste bud cells. *NeuroReport* **12**, 233-235

2) Moriyama *et al.* (2002) Human CAP1 is a key factor in the recycling of cofilin and actin for rapid actin turnover. *J. Cell Sci.* **115**, 1591-1601

3) M. Asano-Miyoshi *et al.* (2001) IP3 receptor type 3 and PLCbeta2 are co-expressed with taste receptors T1R and T2R in rat taste bud cells. *Chemical Senses* **26**, 259-265

269 Poster : Gustatory Transduction

A SINGLE CELL VIEW OF TASTE SENSATION

Huang L.[1], Vo L.[1], Max M.[2], Margolskee R.F.[2] *[1]Monell Chemical Senses Center, Philadelphia, PA; [2]Howard Hughes Medical Institute, Mount Sinai School of Medicine, New York, NY*

Taste sensations are initiated by the interaction of sapid molecules and taste receptor cells in the oral cavity. Taste receptor cells are morphologically and functionally heterogeneous, and utilize multiple transduction pathways for different taste qualities: salty, sour, sweet, bitter and umami. To gain insights into the molecular mechanisms underlying taste sensation, we isolated individual taste receptor cells from taste buds and prepared single taste cell cDNA libraries. To identify differentially expressed genes that might be involved in the distinct functions of different types of taste cells, we used differential screening of the single taste cell cDNA libraries. To gain an overall view of gene expression profiles for these individual taste cells, randomly picked clones from these single taste cell cDNA libraries were also sequenced. Bioinformatic analyses of these cDNA sequences enabled us to categorize these genes as being involved in "housekeeping," taste bud development, taste cell proliferation, differentiation and/or apoptosis, transcription, and signal transduction; we also identified some novel sequences with or without est (expressed sequence tag) matches. The expression of some of these genes in taste cells was verified by in situ hybridization and immunohistochemistry. Further analyses of these single taste cell gene expression profiles could provide novel insights into our understanding of taste sensation, taste plasticity and regeneration. Supported by NIH grants DC05154(LH), DC03155(RFM) and MHS7241(MM). RFM is an Investigator of the Howard Hughes Medical Institute.

270 Poster : Development of Olfactory Systems

ELECTROPHYSIOLOGICAL PROPERTIES OF EMBRYONIC OLFACTORY NEURONS IN EXPLANT CULTURES.

Lischka F.[1], Lamantia A.[2], Rawson N.[1] *[1]Monell Chemical Senses Center, Philadelphia, PA; [2]University of North Carolina, Chapel Hill, NC*

The development of electrical properties in embryonic olfactory neurons (ONs) has not been studied prior to E16 in rodents, when nonselective odor responsive currents can be recorded. We asked whether ONs acquire distinct physiological properties during their initial differentiation from the olfactory placode. We used the perforated patch technique to record from ONs induced in explant cultures of frontonasal epithelium and mesenchyme from E9 embryos*. These cultures recapitulate all known cellular and molecular landmarks associated with ON induction in vivo, and provide a system in which newly induced ONs can be recorded from without complications associated with axotomy or physical disruption that accompany dissection or dissociation from more mature embryos. In these preparations, the neuralized epithelium is readily apparent as a more translucent, slightly invaginated region in the central portion of the explant, surrounded by a denser region of mesenchyme. Stable recordings could be made from cells within this region from explants grown for 48 – 72 hrs. A variety of conductances were observed, including an inward rectifying K+ current, a transient outward K+ current (IA), a voltage-sensitive Ca2+ current and a TTX-sensitive Na+ current. This preparation will enable studies of the development of ion currents leading to odorant sensitivity and the role of mesenchyme-derived signals in this process. Funded in part by DC00214 (NR), DC002876(NR), HD29178 (AL). *LaMantia et al., Neuron, 2000.

271 Poster : Development of Olfactory Systems

UNILATERAL NARIS CLOSURE AND CELL DEATH IN THE NASAL SEPTUM OF THE DEVELOPING RAT

Brunjes P.C.[1], Shurling D.[1] [1]*Psychology, University of Virginia, Charlottesville, Virginia*

Surgically closing one external naris deprives the ipsilateral olfactory epithelium of odorant stimulation and shields it from environmental insult. Several studies have demonstrated that if the procedure is performed on postnatal day one (P1), the ipsilateral olfactory mucosa is 25% thinner by P30. Factors shown to be involved in this change include reduced numbers of immature sensory neurons and slowed cell proliferation. Here we demonstrate that rates of cell death are increased as well. Rats underwent either sham surgery or unilateral naris occlusion on P1 and were killed and perfused 10 (data pending), 20 or 30 days later. Nasal septa were dissected, briefly decalcified in EDTA, and cut in the horizontal plane at 20μm on a crytostat. Tissue was TUNEL-stained using kits from Trevigen. The number of labeled cells found in the deep quarter (proliferative zone), middle half (neural zone), and superficial quarter (sustentacular region) were counted along an average of 2.5 mm and 3.5 mm of tissue/subject in P20 pups and P30 pups respectively. No left/right differences were observed for control pups at any age. In P20 and P30 experimental rats, increased proportions of labeled figures were observed in the neural zone on the closed side. Therefore, naris occlusion thins the olfactory mucosa by reducing cell number, which, in turn, is a product of both slowed cell proliferation and increased cell death. Cell death may result from an inability of young receptors to find adequate trophic support in the olfactory bulb. Supported by DC-00338.

272 Poster : Development of Olfactory Systems

HORIZONTAL BASAL CELLS CONTAIN POTENTIAL STEM CELLS FOR THE OLFACTORY EPITHELIUM

Roskams J.[1], Macdonald J.[1], Murdoch B.[1], Carter L.[1] [1]*Zoology, University of British Columbia, Vancouver, British Columbia, Canada*

In the olfactory neuroepithelium (OE) new olfactory receptor neurons (ORNs) are generated from resident progenitors which reside in the basal cell compartment of the OE. Differing progenitor-like activities have been suggested for both horizontal (HBC) and globose (GBC) basal cells. We have identified combinations of several cell surface adhesion receptors ICAM-1 (CD 54), b-1 Integrin (CD 29), b-4 integrin (CD 104) and the a integrins 1, 3, 6 (CD 49 a, c, f respectively) exclusive to HBCs in vivo. This has enabled us to select individual HBCs by FACS from the mouse OE based on their expression of ICAM-1 (CD54-) and b-1 Integrin (CD 29). CD54+ single HBCs expand rapidly to form colonies; their cloning efficiency is enhanced on a laminin/collagen substrate (mimicking olfactory basal lamina) and by both EGF and TGFa. Colony expansion is enhanced by EGF, TGFa and LIF. Differentiated HBC Colonies contain expanded HBC and globose basal cell (GBC) populations, and progeny that resemble both olfactory neuronal and glial lineages. HBCs thus display a shared primitive stem-like state (based on integrin expression) with stem cells of other lineages, self-replicate and can produce multiple differentiated progeny for the OE. In addition, we have isolated a hoechst-negative side population (SP) of cells from the postnatal mouse OE that resembles the SP fraction found in mesenchymal, muscle and haematopoietic stem cell populations. We are currently testing whether this population is an HBC, or represents a potentially different progenitor subcompartment, within the OE. This work is supported by a grant from the CIHR (TOP37542) and NIH (3RO1 DC04579-02S1).

273 Poster : Development of Olfactory Systems

DYNAMIC EXPRESSION OF DEVELOPMENTAL GENES IN THE ADULT ANTENNA OF *MANDUCA SEXTA*

Bohbot J.D.[1], Vogt R.G. [1]*Biological Sciences, University of South Carolina, Columbia, SC*

The morphology of the antenna consists of a succession of annuli, ventrally covered by olfactory units or sensillae arranged in a specific pattern. We looked at the temporal expression of three developmentally regulated genes in the sphingid moth *Manduca sexta*: *Distal-Less* (DLL), *Notch* (N) and *Pupal-Cuticle Protein* (PCP). The adult antenna forms from a final larval instar antennal imaginal disc; the disc everts at pupation and neurogenesis and morphogenesis follow. This study was conducted in the late antennal imaginal disc of the final instar larva and in the early developing adult antenna. DLL is continually expressed throughout appendage (e.g. antenna) development, establishing proximodistal pattern elements. Vertebrates DLL homologues are involved in olfactory development. N is involved in neurogenesis by shaping the pattern of the nervous system. N is also required for the segmentation and growth of both leg and antenna. PCP, a stage specific gene, contributes to the formation of the pupal cuticle in *Galleria mellonella*. We have partially cloned these three genes and used Real-Time PCR to characterize their expression levels during the early stages of metamorphosis. Their pattern of expression is consistent to previous studies done in *Drosophila* and *Galleria*. DLL and N display a bimodal expression pattern: they are first strongly expressed during antennal imaginal disc growth and again later during neurogenesis at lower levels. Like in *Galleria*, a strong PCP expression level marks the onset of the new developmental program that is taking place during the larval-pupal transition.

274 Poster : Development of Olfactory Systems

THYROID HORMONE-REGULATED NOSE GENES DURING *XENOPUS LAEVIS* DEVELOPMENT

Walworth E.[1], Burd G.D.[1] [1]*Molecular and Cellular Biology, University of Arizona, Tucson, Arizona*

The nose of young *Xenopus* tadpoles contains two areas of sensory epithelium. At metamorphosis, one of these areas is completely remodeled and a new area of sensory epithelium is formed *de novo*. The goal of our current study is to identify the genes regulated by thyroid hormone (TH) that are involved in remodeling the olfactory epithelium at metamorphosis. We used representational difference analysis (RDA) to identify direct and early response genes that are up- and down-regulated by TH in the nose of the African clawed frog, *Xenopus laevis*. The RDA mRNA sources were from wild-type stage 46/47 tadpoles and stage 46/47 tadpoles treated for 48 hours with 100nM T_3 (3,3',5-triiodo-L-thyronine) (Brown, et al 1996 and Y. Shi, pers. comm.) Following three rounds of subtraction, all gene products were cloned, sequenced and subjected to TIGR and NCBI BLAST database searches identifying 11 unique up-regulated and 29 unique down-regulated gene fragments. In a prior RDA surveying genes expressed at metamorphic climax, predominately cytoskeletal, membrane, extracellular matrix and intermediary metabolism genes were found. In this experiment with short term TH treatment, genes for cell growth control and transcriptional activation and regulation predominate, including thyroid hormone receptor β. TRβ genes are regulated in a cell-type specific manner that correlate with tissue transformation, participating both in inducing larval epithelial apoptosis and stimulating adult cell proliferation (Shi and Ishizuya-Oka 1997). Both apoptosis and cell proliferation are essential for olfactory epithelium remodeling at metamorphosis. Support: NIDCD #DC03905

275 Poster : Development of Olfactory Systems

GLIA IN THE AXON SORTING ZONE OF THE MOTH PRIMARY OLFACTORY PATHWAY ALTER AXON RESPONSES TO SUBSEQUENT GLIAL ENCOUNTERS.

Oland L.A.[1], Howard C.T.[1], Tolbert L.P.[1] [1]*Arizona Research Laboratories Division of Neurobiology, University of Arizona, Tucson, Arizona*

Olfactory receptor neurons (ORNs) navigating toward termination sites in the antennal (olfactory) lobe in the moth *Manduca sexta* encounter two populations of glial cells, the first as they enter the sorting zone (SZ), the most proximal region of the antennal nerve, and the second as they grow through the rind of glial cells that surrounds the antennal-lobe neuropil. Previous studies have shown a dramatic change in direction of growth, in fasciculation, and in growth cone complexity as the axons traverse the SZ. Within the glial rind, ORN axons typically show much less marked changes. Yet in cultures in which ORN axons contact glial cells, both types of glia elicit similar increases in growth cone size and complexity (E. Tucker, PhD dissertation, 2002). This raises the possibility that the differences in ORN axon responses obtained *in vivo* and *in vitro* occur because, *in vivo*, exposure of the axons to SZ glia attenuates or alters their subsequent response to the neuropil glia. In the current study, we cultured slices of the antennal lobe with antennal explants (ORNs) to test this hypothesis. We placed antennal explants adjacent to the antennal-lobe slice so that ORN axons were forced to encounter neuropil glia without first encountering SZ glia. Preliminary results suggest that these "naive" growth cones are similar in size and shape to those seen in SZ slices, consistent with the previous *in vitro* results and suggesting that SZ glia may alter axonal responsivity to neuropil glia. Supported by NS28495 and NS20040.

276 Poster : Development of Olfactory Systems

INTEGRINS IN THE DEVELOPING OLFACTORY SYSTEM

Whitley M.K.[1], Treloar H.B.[1], De Arcangelis A.[2], Georges Labouesse E.[2], Greer C.A.[1] [1]*Neurosurgery, Yale University, New Haven, CT;* [2]*Institut de Genetique et de Biologie Moleculaire et Cellulaire, CNRS/INSERM/ULP, Strasbourg, France*

Integrins are heterodimeric cell surface receptors that may mediate developmental events by binding ECM ligands. *In vitro*, neuronal migration and neurite outgrowth from olfactory epithelium is mediated in part by integrin α6β1 interactions with laminin (Calof, et al. 1991, 1994). We intend to characterize the *in vivo* expression and possible function of integrin α6 in the development of the olfactory bulb (OB) by using an antibody specific to the α6 subunit to examine expression from embryonic (E) day 13 to postnatal (P) day 4 in CD-1 mice. In addition, we are studying OB organization in E17 α6 knockout (KO) mice. In CD-1 mice from E13 to E17, α6 localizes in a radial pattern extending from the core of the OB to the nerve layer. By PO, expression is limited to the external plexiform layer (EPL) and olfactory ensheathing cells (OEC) where it colocalizes with laminin and p75. In the α6 KO mice laminin staining is attenuated in OEC's, though NCAM staining of sensory neuron axons appears normal. However, MAP2 staining suggests that the laminar organization of the OB is perturbed in the KO mice, perhaps due to the aberrant formation of dendritic processes. These results suggest that integrin α6 may play important roles in establishing the neuronal and synaptic organization of the OB during development. Supported in part by NIH DC00210, DC03887, and NS10174. M.K. Whitley is a Howard Hughes Medical Institute Medical Research Training Fellow.

277 Poster : Development of Olfactory Systems

ASYCHRONOUS DEVELOPMENT OF OLFACTORY SENSORY PROJECTIONS

Zou D.[1], Feinstein P.[2], Greer C.A.[3], Mombaerts P.[4], Firestein S.[1] [1]*Biological Sciences, Columbia University, New York, NY;* [2]*Mombaerts Lab, Rockefeller University, New York, NY;* [3]*Neurosurgery, Yale University, New Haven, CT;* [4]*Laboratory of Vertebrate Developmental Neurogenetics, Rockefeller University, New York, NY*

The processes involved in establishing and maintaining olfactory glomeruli have been investigated with gene-targeted mice in which specific populations of olfactory sensory neurons, expressing a given odorant receptor (OR) gene, are visualized. The M71 and M72 ORs are highly homologous and their glomeruli are near each other. In young gene-targeted mice, multiple glomeruli in variable positions were observed at the two conserved lateral and medial sites in the bulb. In contrast, most mature mice had a single glomerulus at both sites, indicating that glomerular formation undergoes robust remodeling. We then examined whether sensory input levels influence this OR specific glomerular remodeling. Unilateral naris occlusion at P0 led to multiple M71 or M72 glomeruli at both lateral and medial sites in most ipsilateral bulbs, and had no effect on the number of M71 or M72 specific glomeruli in the contralateral bulbs. When occluded at P15, extra M72 glomeruli were no longer observed. However, extra M71 glomeruli were still apparent in many occluded mice, suggesting that the remodeling of glomeruli occurs within variable critical periods depending on the OR. Taken together our results suggest that glomerular formation is asynchronous, that active remodeling occurs at certain OR specific glomeruli, and that afferent sensory activity differentially modulates glomerular remodeling within critical periods specific for individual ORs.

278 Poster : Development of Olfactory Systems

TENASCIN-C IS AN INHIBITORY GUIDANCE MOLECULE IN THE DEVELOPING MOUSE OLFACTORY BULB

Treloar H.B.[1], Yang Y.[1], Greer C.A.[1] [1]*Neurosurgery, Yale University, New Haven, CT*

During development olfactory sensory neurons (OSNs) extend axons to the telencephalon and induce the formation of the olfactory bulb (OB). After axons reach the telencephalon they form a presumptive olfactory nerve layer (pONL), directly apposed to a dense meshwork of developing mitral/tufted (M/T) cell dendrites. Axons remain restricted to the pONL for up to 4 days before growing in deeper into the OB and inducing glomerular development. Likewise, developing (M/T) dendrites remain restricted to the dendritic zone and do not grow into the pONL. Which cue(s) are present in the developing OB that prevent premature intermingling of axons and dendrites? One candidate inhibitory molecule is the extracellular matrix molecule tenascin-C (TNC). TNC is oligomeric glycoprotein which 1) is highly expressed in developing CNS; 2) is downregulated in adult CNS; 3) is growth modulatory to many neuronal populations *in vitro*; 4) has multiple receptors; and 5) is expressed in boundary-like patterns elsewhere in the CNS. We examined expression of TNC in the developing olfactory system and found it highly expressed in the OB, deep to the pONL. *In vitro*, purified TNC inhibits neurite outgrowth from OSN explants. Moreover, dissociated M/T neurons grown on patterned substrates avoid TNC boundaries. These data provide strong evidence that TNC is an inhibitory guidance cue to both OSN axons as well as M/T dendrites in the developing OB. Interestingly, TNC expression remains high in the OB during the postnatal period. Preliminary analyses of TNC-receptor expression reveal dynamic expression patterns in different neuronal populations, suggesting that responses to TNC are modulated at the receptor level. Supported by DC00210 to CAG.

279 Poster : Development of Olfactory Systems

CADHERINS AND CATENINS: EXPRESSION IN MOUSE OLFACTORY PATHWAY DEVELOPMENT

Akins M.R.[1], Greer C.A.[2] [1]*Interdepartmental Neuroscience Program, Yale University, New Haven, Connecticut;* [2]*Neurosurgery, Yale University, New Haven, Connecticut*

Olfactory sensory neurons (OSNs) extend axons to the olfactory bulb (OB), where they target glomeruli and form synapses with remarkable specificity. Studies have indicated that odorant receptors are necessary but insufficient to fully account for correct targeting, suggesting that other guidance molecules are involved. Members of the cadherin family are good candidates for contributing to the specificity of OSN targeting and synapse formation. 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 axon guidance and formation of synaptic circuits. A PCR screen demonstrated that E-Cadherin (CDH1) and N-Cadherin (CDH2) are expressed in the olfactory system throughout development. Analysis of protein expression via Westerns shows that these proteins are most highly expressed during embryonic and early postnatal periods, with levels declining thereafter. Using immunohistochemistry, both CDH1 and CDH2 were localized to the developing olfactory nerve. Both β- and γ-catenin (two mutually exclusive binding partners of the cadherins) localize to the nerve, where they may mediate intercellular adhesion. In contrast, only CDH2 and β-catenin are present in OB glomeruli and external plexiform layer, where they may contribute to synaptic adhesion. These data provide evidence for cadherin involvement in the development of the primary olfactory pathway. Supported in part by DC00210, DC03887, and NS10174 to CAG.

280 Poster : Development of Olfactory Systems

MICROARRAY ANALYSIS OF BONE MORPHOGENETIC PROTEINS (BMPS), RECEPTORS AND MODULATORS IN THE DEVELOPING MOUSE OLFACTORY BULB (OB)

Ukhanova M.[1], Margolis J.[1], Margolis F.L.[1] [1]*Anatomy & Neurobiology, University of Maryland, Baltimore, Maryland(MD)*

BMPs, members of the TGF-B superfamily of growth factors, participate in development, morphogenesis, cell proliferation and apoptosis. The BMP signal transduction pathway: 1-binding to specific ser/thr kinase receptors, 2-signal transmission to the nucleus via Smad proteins and 3-their association with specific DNA-binding proteins leads to target-specific gene expression. BMP activities are modified by interaction with several secreted proteins, e.g. chordin, noggin, BAMPI. We reported the differential expression of BMP4, 6, 7 in the developing olfactory system (Peretto et al. JCN 2002). Others have reported differential expression of BMPs and BMP-receptors in pre- and post-natal CNS. We are using a 96 gene array to study the expression of members of this pathway during OB ontogeny. We observed that the the level of noggin (ng) expression in (+/+) mice is ca.3X that in (+/-) ng-KO mice verifying the method and consistent with the expected 2X difference in ng expression between the (+/+) and (+/-). We have identified a few other genes whose expression appears to differ between the (+/+) vs. the (+/-) ngKO indicating that expression of other genes responds to a 50% ng reduction. We have confirmed some of the differences by RT-PCR. We are now evaluating changes in OB and cerebellum during postnatal ontogeny. The mRNA expression profiles in OB and cerebellum differ and there are significant ontogenetic changes in these profiles in OB. This method will facilitate identification of those mRNAs most responsive to ontogeny and lesion/regeneration for further study. Supported in part by NIH DC00347 and DC03112.

281 Poster : Development of Olfactory Systems

NEURITE STABILITY IN THE MOUSE OLFACTORY SYSTEM: THE ROLE OF NOGO, P75, & RHOA.

Iwema C.L.[1], Strittmatter S.M.[2], Greer C.A.[1] [1]*Neurosurgery, Yale University, New Haven, Connecticut;* [2]*Neurobiology, Yale University, New Haven, Connecticut*

Olfactory sensory neurons (OSNs) continually regenerate and extend axons that reinnervate specific targets in the olfactory bulb (OB). As they navigate the nerve layer, OSN axons are unbranched until they reach their synaptic partners, the mitral/tufted cells (M/T). The dendritic arbors of M/T cells remain remarkably stable considering the continuous turnover of both the OSNs and interneurons of the OB. What are the molecular cues that: (1) influence OSN axons to migrate unbranched and (2) reinforce the stability of M/T cell dendritic arbors? Recent data from the spinal cord suggest that the inhibitory molecules Nogo and MAG govern axon growth via their interaction with both Nogo Receptor (NgR) and p75 neurotrophin receptor. This receptor complex transmits its signal through the Rho family of proteins, regulating axon-glial interactions, neurite branching, and dendritic stability. To test the hypothesis that these regulatory molecules are involved in the development and maintenance of the olfactory system, we demonstrated their presence in the olfactory epithelium and OB of CD-1 mice with immunohistochemistry, ultrastructural localization, and Westerns. Antibodies included: NgA, MAG, NgR, p75, RhoA, p190 RhoGAP, NCAM, S100. Consistent with a role in olfactory system development, in primary cultures of OB neurons, axon outgrowth is augmented by blocking antibodies/peptides (NEP1-40, NGFR5, p190 RhoGAP) but inhibited with the addition of RhoA. Thus, in the olfactory system the NgR/p75 complex appears to mediate neurite outgrowth/stability by influencing cytoskeletal dynamics. Supported by NIH:NS10174.

282 Poster : Salt & Sour Taste

CHARACTERIZATION OF AMILORIDE-INSENSITIVE SODIUM SALT RESPONSES USING PATCH CLAMP RECORDING.

Klein J.T.[1], Burks C.A.[1], Gilbertson T.A.[1] [1]*Biology, Utah State University, Logan, UT*

The transduction of sodium salts has been shown to involve both amiloride-sensitive (A-S) and -insensitive (A-I) pathways. While the former pathway is reflective of Na^+ movement through the epithelial sodium channel (ENaC), the molecular underpinnings of the A-I pathway have remained elusive. Recently, a cetylpyridium Cl (CPC)-sensitive conductance (DeSimone *et al. J. Neurophysiol* 86:2638, 2001) and a Na^+-sensitive G protein coupled receptor that leads to K channel closure (Ikeda *et al. Soc. Neurosci. Abs.* #355.16, 2002) have been hypothesized to mediate the A-I response. To examine this we have performed patch clamp recording on rat taste receptor cells (TRCs) from the three lingual papillae to look for direct evidence of these pathways. We tested CPC (0.005 – 25 μM) on TRCs in the whole-cell and perforated patch configuration. Though these concentrations were several orders of magnitude less than applied to the tongue previously, concentrations above 5 μM tended to disrupt cell membranes, which may reflect the detergent/antiseptic action of CPC. Concentrations of 5 μM and below produced equivocal results; Na^+ currents were enhanced in some cells and inhibited in others. Interestingly, few TRCs responded to both amiloride and CPC; thus CPC could not completely account for the A-I response. To test for the involvement of K^+ channel inhibition, we monitored the TRC K^+ conductance during changes from a Na^+-free to Na^+-containing solution. We saw no effects of Na^+ on outward K^+ currents in these experiments. Our results suggest additional unidentified mechanisms must contribute to the A-I Na^+ responses in rat TRCs. Supported by NIDCD 002507.

283 Poster : Salt & Sour Taste

IDENTIFICATION OF NATRIFERIC HORMONE RESPONSIVE ELEMENTS IN TASTE CELLS: IMPLICATIONS FOR THE REGULATION OF SALT & WATER TASTE.

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

Previous studies have demonstrated that salt-transduction pathways involving the epithelial sodium channel (ENaC) are responsive to natriferic hormones like aldosterone (ALDO) and vasopressin (AVP). To explore the plasticity of the peripheral taste system in greater detail, we have identified a variety of ion channels and intermediates that are known to be sensitive to ALDO and AVP in other transporting epithelia. Using RT-PCR, we have identified a number of proteins in taste buds that have been shown to respond to AVP and/or ALDO. In addition to ENaC, we have identified the hormone-sensitive renal outer medullary K^+ channel (ROMK1) and the Cl^- channel, ClC-2, which are involved in K^+ and Cl^- homeostasis, respectively. Taste buds also contain the cystic fibrosis transmembrane conductance regulator (CFTR), which in addition to being regulated by ALDO, also directly regulates ENaC-mediated conductances. AQP-2, which we have also identified in taste cells by RT-PCR and immunocytochemistry, is the vasopressin-regulated water channel of the kidney. Finally, we have attempted to identify intermediates in the pathway underlying hormone-regulated changes in ENaC. Taste cells express NEDD-4, a regulatory protein that controls ENaC expression by promoting its ubiquitination and degradation. The ALDO-induced immediate early gene sgk (serum- and glucocorticoid-regulated kinase), which leads to an increase in ENaC-mediated currents by inhibiting NEDD-4, is highly expressed in all taste bud types. Implications of these findings for the regulation of salt and water taste will be discussed. Supported by NIDCD 02507.

284 Poster : Salt & Sour Taste

HAMSTER CHORDA TYMPANI RESPONSES TO POTASSIUM ARE VOLTAGE-SENSITIVE

Edgar B.[1], Proko E.[1], Stewart J.[1], Stewart R.[1] [1]*Psychology/Program in Neuroscience, Washington and Lee University, Lexington, VA*

We studied the voltage sensitivity of hamster chorda tympani (CT) taste responses to potassium chloride (KCl) and potassium gluconate (KGlu). In rat, CT responses to potassium salts show little sensitivity to receptive field voltage perturbation. Also in rat, potassium salts with large anions evoke small, slow onset CT nerve responses with high threshold concentrations. We expected hamster CT responses to KCl and KGlu to behave similarly to those seen in rat. Multiunit CT responses to 50 and 500 mM KCl and KGlu were obtained during receptive field voltage clamp from anesthetized hamsters. Stimulus-evoked responses were recorded and analyzed offline. The dynamics of open circuit chorda tympani responses to both KCl concentrations were similar to those in rat. Specifically a large, transient response component was followed by a lower steady state component. Likewise, CT responses to both 50 and 500 mM KGlu showed pronounced onset transients followed by lower steady state components. This observation differs considerably from previous findings in rat. Transepithelial voltage clamp modulated CT responses to these salts, with the greatest effect seen at 500 mM for both KCl and KGlu. One-tailed t-tests of voltage sensitivity indices (i.e., the difference between negative and positive voltage clamp response magnitudes for a given stimulus concentration) revealed that the voltage sensitivity of responses to both 50 and 500 mM KCl and KGlu was statistically significant ($ps < 0.04$). These results suggest that in hamster potassium ions are transduced via an apical membrane conductance. Support: NIH-DC04734

285 Poster : Salt & Sour Taste

VOLTAGE-SENSITIVITY OF HAMSTER CHORDA TYMPANI SODIUM RESPONSES

Proko E.[1], Greene R.[1], Russell O.[1], Hatzis C.[1], Spatzer R.[1], Stewart R.[1] [1]*Psychology/Program in Neuroscience, Washington and Lee University, Lexington, VA*

We examined the voltage sensitivity of hamster chorda tympani (CT) responses to sodium (Na) salts containing anions of varying size. Chorda tympani responses to concentration series (10-500 mM) of sodium chloride (NaCl) and sodium gluconate (NaG) were obtained from anesthetized adult hamsters during simultaneous lingual epithelial voltage clamp. Responses to NaCl and NaG behaved similarly under voltage clamp. Even at high concentrations, responses to NaCl retained significant voltage sensitivity ($ps < 0.05$). This differs from previous observations in rat. Response data were also fit with the apical channel model to determine aggregate kinetic behavior of the apical Na sensing apparatus. Best-fit parameters for estimated maximum CT response (an analog of apical channel density) and δ (fraction of clamp voltage dropped across apical Na channels) were very similar for both NaCl and NaG. Estimated CT maxima were 1.44 (NaCl) and 1.54 (NaG). Estimated δs were 1.00 (NaCl) and 0.94 (NaG). On the other hand, the estimated Michaelis constants for the two salts differed considerably (63.1 mM for NaCl versus 252 mM for NaG). Together, these results imply that the paracellular shunt pathway in the adult hamster is especially impermeant, and that the majority of the CT response to Na salts in hamster derives from a voltage-sensitive, apical transduction mechanism. However, even a small anion-dependent paracellular shunt conductance seems to promote field potentials large enough to modulate apparent apical Michaelis constants strongly. Supported by NIH-DC04734

286 Poster : Salt & Sour Taste

ALTERATION OF THE TASTES OF SALTS BY CATHODAL CURRENT

Do D.[1], Su H.[1], Frank M.E.[1], Hettinger T.P.[1] [1]*Neurosciences, Dept. Oral Diagnosis, UConn Health Center, Farmington, CT*

Electrical currents alter tastes of salts in humans. Anodal (+) currents produce metallic tastes but little is known about effects of cathodal (-) currents (Nguyen et al., unpublished). In 2 studies, effects of cathodal currents on tastes of moderately intense concentrations of 4 chloride salts (NaCl, KCl, NH_4Cl, $CaCl_2$) and 4 sodium salts (NaCl, Na Acetate, $NaNO_3$, Na Saccharin) were examined. An electrogustometer provided currents (0μA, -40μA, -80μA) through salt solutions in a tube or cup. With the cathode contacting solutions, subjects (N=10-12, mean age 25.2 yr.) held the anode and completed circuits by placing tongue tips into solutions. Ratings of intensity (on a 10-point, fixed-interval scale) and quality identification before, during, and after 2-3s current applications were made. Data were analyzed by ANOVA, $\alpha = 0.05$. Compared to 0μA, total taste intensities of chloride salts were 25% lower during -40μA currents. Reductions were no greater for -80μA. At 0μA, the chloride salts were most frequently identified as salty-bitter, attributions 25% less frequent for -40 and -80μA currents, suggesting salty and bitter sensations are driven by cations. When quality-specific intensities of sodium salts were rated, -80μA current lowered totals for NaCl and $NaNO_3$ by 33%, decreasing both salty and sour ratings. Rating totals for Na Acetate and Na Saccharin were unaffected, suggesting inhibitory effects of cathodal current are reduced when anions contribute to the taste. Thus, where anodal current enhances cation-dependent tastes, cathodal current inhibits them. [Supported by NIH: T35 DE07136]

287 Poster : Salt & Sour Taste

EXPRESSION OF ACID SENSING ION CHANNELS IN ISOLATED HUMAN TASTE RECEPTOR CELLS

Huque T.[1], Lischka F.[1], Spielman A.I.[2], Bayley D.L.[1], Cao J.[1], Feldman R.S.[3], Brand J.G.[1] *[1]Neuroscience, Monell Chemical Senses Center, Philadelphia, PA; [2]College of Dentistry, New York University, New York, NY; [3]Dental Medicine, V.A. Medical Center, Philadelphia, PA*

We have previously reported the cloning and functional expression of an acid sensing ion channel (ASIC1a) from human fungiform papillae that acts as a sourness detector. An important question left unanswered by these studies was whether the mRNA for this molecule is located in human taste receptor cells. To address this issue we isolated human taste receptor cells, and also nonsensory lingual epithelial cells, from fungiform papillae obtained from volunteers by surgical biopsy. Six separate groups of 5-15 taste or nonsensory cells were collected into tubes containing fifty microliters of RNAlater™. A seventh tube contained a single taste bud. RTPCR was then performed, followed by subcloning and sequencing of the PCR products. Using this approach we confirmed the presence of mRNA for ASIC1a in all groups of human taste receptor cells that were examined, including the taste bud preparation, and also in one group of nonsensory epithelial cells. The mRNA for ASIC2a (a sourness detector in rat circumvallate papillae) was expressed in two groups of human taste cells, including the taste bud, and in one group of epithelial cells. The mRNA for ASIC-beta (a mediator of sour taste in rat fungiform papillae) was expressed only in the taste bud. ASIC3 was not detected in any cells. Immunohistochemical data confirmed the presence of ASIC1a and ASIC-beta protein in taste buds of human fungiform papillae. Supported in part by a Dept of Veterans' Affairs grant (JGB); and BARD Grant #IS-2518 (AIS).

288 Poster : Salt & Sour Taste

ACID-INDUCED CALCIUM RESPONSES IN MURINE TASTE CELLS

Richter T.A.[1], Pereira E.[1], Roper S.D.[2] *[1]Physiology & Biophysics, University of Miami School of Medicine, Miami, FL; [2]Physiology & Biophysics and Program in Neuroscience, University of Miami*

Sour taste is caused by H^+ ions released by acids. Several types of acid-sensitive ion channels have been proposed as sour taste transducers, including members of the degenerin/epithelial (MDEG/ENaC) and hyperpolarization-activated cyclic nucleotide-gated (HCN) channel families. Because sour taste perception and expression of MDEG/ENaC and HCN channels in taste cells have not been directly correlated, the identity of the sour tansducer(s) in mammalian taste cells is unresolved. Many of the recently cloned 14-member family of two pore domain potassium (KCNK) channels are sensitive to acidification and, therefore, are potential candidates for transducing sour taste. In this study, we recorded the responses of murine taste cells to citric acid, using Ca^{2+} imaging of intact taste buds in slices of lingual tissue. We systematically examined the effect on Ca^{2+} responses of a battery of pharamacological treatments that have been shown to affect KCNK channels. Ca^{2+} responses of taste cells to citric acid were inhibited by Ba^{2+} (2 mM), Gd^{3+} (500 μM), halothane, Zn^{2+} (1 mM) and cold. Responses were unaffected by 4-AP (1 mM), anandamide (50 μM), arachidonic acid (100 μM), Cs^+ (500 μM), quinidine (200 μM), quinine (1 mM), riluzole (500 μM) and TEA (10 mM). This response profile is remarkably similar to that of recombinant TWIK-1 (KCNK1) and TWIK-2 (KCNK6) channels and also resembles the responses of TREK-1 (KCNK2) and TREK-2 (KCNK10). In addition, mouse taste cells were strongly immunopositive for TREK-1 but not for TASK-1 (KCNK3) or TRAAK (KCNK4). These results suggest that members of the KCNK family of ion channels, particularly TWIK-2 and TREK-1, are potential candidates for sour taste transduction in mammals. Supported by NIDCD/NIH grant #DC00374.

289 Poster : Salt & Sour Taste

RELATIONSHIP BETWEEN INTRACELLULAR PH AND CA2+ IN FUNGIFORM RAT TASTE RECEPTOR CELLS.

Lyall V., Malik S.A., Alam R.I., Heck G.L., Desimone J.A. *VCU, Richmond, VA*

In TRCs an acid-induced decrease in pH_i is a proximate stimulus for sour taste transduction. However, the subsequent intracellular signaling events for sour taste transduction are not known. Since an increase in $[Ca^{2+}]_i$ is required for transmitter release from TRCs to taste nerves, we investigated the relationship between pH_i and $[Ca^{2+}]_i$ by direct measurement of pH_i and $[Ca^{2+}]_i$ in polarized FF TRCs. TRCs were loaded with the pH-sensitive dye BCECF or with the Ca^{2+}-sensitive dye Fura-2. Temporal changes in pH_i and $[Ca^{2+}]_i$ were monitored using ratio imaging while the apical or basolateral membranes of TRCs were stimulated with strong acids or weak organic acids at constant pH. A unilateral decrease the apical pH from 7.4 to 3.0 with citric acid, acetic acid or HCl decreased resting pH_i and increased $[Ca^{2+}]_i$. At constant extracellular pH (7.4), exposing the basolateral membrane to 15 mM Na-acetate transiently decreased TRC pH_i and increased $[Ca^{2+}]_i$ followed by spontaneous pH_i and $[Ca^{2+}]_i$ recovery to baseline. Upon Na-acetate washout pH_i increased transiently with a concomitant decrease in $[Ca^{2+}]_i$. This was followed by slow recovery of pH_i and $[Ca^{2+}]_i$ towards baseline. In contrast, exposing the basolateral membrane to 15 mM NH_4Cl alkalinized TRC pH_i and decreased $[Ca^{2+}]_i$. Upon NH_4Cl washout, pH_i became acidic with a decrease in $[Ca^{2+}]_i$. We conclude that a decrease in pH_i is followed by an increase in $[Ca^{2+}]_i$, a necessary precursor to transmitter release by acid-sensing TRCs. Supported by NIDCD grants DC-02422 and DC-00122.

290 Poster : Salt & Sour Taste

LINGUAL SURFACE POTENTIAL (LSP) IN HUMANS

Feldman G.M., Heck G.L., Santos C., Clary R., Lyall V., Desimone J.A., Mogyorosi A. *Virginia Commonwealth University, Richmond, VA*

Salt sensing in animals involves the epithelial sodium channel (ENaC). If ENaC were involved in human salt sensing, then the LSP would hyperpolarize when exposed to sodium, a dose response relationship would exist between the LSP and the Na concentration, and amiloride, an inhibitor of ENaC, would block Na induced hyperpolarization. We developed a chamber to measure the LSP while different solutions superfused the surface of the tongue and a technique to adjust for the junction potentials induced by varying salt concentrations. Changing the superfusion solution from rinse solution (30 mM KCl) to 300 mM NaCl (+30 mM KCl) caused the LSP to hyperpolarize by 10.1 ± 0.7 mV ($n=13$, $p<0.001$) over 30 seconds. With repeated challenge the LSP response was reproducible. Increasing the Na concentration from 100 mM to 600 mM increased the LSP from 7.5 ± 0.6 mV to 10.3 ± 1.1 mV ($n=9$, $p<0.001$). To examine whether amiloride affects the LSP 0.1 mM amiloride was added to 300 mM NaCl. Amiloride reduced the hyperpolarization by 1.9 ± 0.6 mV ($n=9$, $p<0.01$). However, it was striking that the amiloride effect was not uniform: in 5 volunteers amiloride inhibited the LSP by as much as 42%, while in 4 subjects amiloride inhibited <5% of the LSP. In an amiloride sensitive volunteer, amiloride exerted 50% of its effect at 1 μM. In conclusion we have demonstrated that the LSP can be measured in humans, that Na hyperpolarizes the LSP, that increasing the Na concentration increases LSP hyperpolarization, and that amiloride inhibits the Na evoked LSP in some humans. While ENaC is involved in sensing salt, its role appears to vary amongst individuals. We speculate that ENaC involvement in the LSP may vary because of genetic control and physiological conditions.

291 Poster : Umami Taste

IMMUNODETECTING A CANDIDATE UMAMI RECEPTOR, TASTE-mGLUR4, IN TASTE CELLS

Chaudhari N.[1], Pereira E.[2], Landin A.M.[2], Roper S.D.[1] *[1]Physiology & Biophysics and Program in Neuroscience, University of Miami, Miami, Florida; [2]Physiology & Biophysics, University of Miami, Miami, Florida*

Candidate taste receptors for glutamate (i.e. *umami* receptors) have been cloned and expressed in functional assays (Chaudhari et al 2000; Nelson et al 2002; Li et al 2002). These receptors include a truncated variant of a metabotropic synaptic glutamate receptor, taste-mGluR4, and a dimer of T1R1 with T1R3. Although receptor mRNAs have been detected in specific taste bud cells using *in situ* hybridization, the corresponding receptor proteins have previously been difficult to visualize. We raised antibodies against C-terminal and N-terminal peptides in the taste-mGluR4 sequence. In immunoblots, the antibodies stained only bands of the expected molecular weight from HEK293 cells transfected with taste- or brain-mGluR4. In taste papillae from rat and mouse, we detected a protein band of approx. 70kDa, corresponding to taste-mGluR4. This band was either absent or present at much lower concentration in non-taste lingual samples. In immunohistochemistry, the antibodies robustly immunostained HEK293 cells expressing taste- and brain-mGluR4, but did not react with untransfected HEK293 cells. The same antibodies also strongly immunostained a subset of cells in vallate, fungiform and pallatal taste buds from mice and rats. These data, obtained with validated antibodies, support the conclusion that taste-mGluR4 is expressed in a limited subset of vallate, fungiform and palatal taste bud cells. Double-label immunohistochemistry is in progress to determine which G protein subunits are co-expressed with mGluR4 in taste cells. Supported by NIDCD/NIH grant 1PO1 DC03013.

292 Poster : Umami Taste

RESPONSES TO GMP AND GLUTAMATE IN TASTE RECEPTOR CELLS OF RAT FUNGIFORM PAPILLAE

Ogura T.[1], Lin W.[2], Kinnamon S.C.[1] *[1]Biomedical Sciences, Colorado State University, Fort Collins, CO; [2]Cell & Structural Biology, University of Colorado Health Sciences Center, Denver, CO*

The 5′-ribonucleotide guanosine 5′-monophosphate (GMP) is used widely as an umami taste stimulus and a potent flavor enhancer as it synergistically increases the umami taste elicited by monosodium glutamate (Glu). Transduction mechanisms for GMP itself and its synergy with glutamate are largely unknown. Here, we examined responses to GMP, Glu, and a mixture of both in taste receptor cells of rat fungiform papillae using whole-cell patch-clamp and Ca^{2+} imaging. In whole-cell current recordings, GMP induced response types that are similar to those of Glu. In Ca^{2+} imaging, GMP alone, as well as Glu alone and a mixture of both, increased intracellular Ca^{2+} levels. Most of the GMP-responsive cells were responsive to Glu. Interestingly, some taste cells can respond only to GMP or Glu, indicating that GMP and Glu likely activate different receptors in those cells. The mixture of GMP and Glu induced larger responses than the summation of individual responses to GMP and Glu in a subset of cells. Further, more cells responded to the mixture than to the individual stimuli. Therefore, synergistic taste responses elicited by the mixture of GMP and Glu could be due both to a potentiated intensity of response and an increased number of responding cells. Supported by NIH grant DC03013 to SCK.

293 Poster : Umami Taste

CAN RATS DISCRIMINATE BETWEEN THE TASTES OF IMP, GMP AND MONOSODIUM GLUTAMATE?

Faes T.M.[1], Wifall T.C.[1], Mitzelfeld J.D.[1], Padillo A.M.[1], Delay E.R.[1] *[1]Neuroscience Program, Regis University, Denver, CO*

Monosodium glutamate (MSG), found in protein-rich foods such as fish, cheeses, and vegetables, is defined by its unique taste called *umami* and its ability to interact synergistically with inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate (GMP). Conditioned taste aversion studies report that an aversion to MSG generalizes strongly to IMP and GMP, indicating that IMP and GMP elicit an umami-like taste (Ninomiya & Funakoshi, *Physiol. & Behav.*, 1989; Yamamoto et al., *Physiol. & Behav.*, 1991). However, knowledge of the specific taste qualities of IMP and GMP is limited and almost always linked to studies of MSG or other amino acids. We wanted to determine 1) detection thresholds and recognition thresholds for IMP and GMP in rats, and 2) if rats could discriminate between the tastes of MSG and IMP. Detection thresholds for IMP and GMP (approximately 0.0001 mM) were well below thresholds for MSG. Recognition thresholds for IMP and GMP were between 0.1-.5 mM. In discrimination experiments, saliency of Na^+ in MSG solutions was reduced by adding 30 μM amiloride to all solutions and equimolar NaCl concentrations to IMP. Rats reliably discriminated between MSG (10-150 mM) and low concentrations of IMP (0.01-1 mM) but were less proficient when IMP was greater than 1 mM. Similar discrimination experiments with GMP and MSG are being conducted. These data indicate multiple receptor types are involved in the taste of umami that may include a taste-mGluR4 receptor (Chaudhari et al., *Nature Neurosci.*, 2000) and/or an amino acid receptor (Nelson et al., *Nature*, 2002). Supported by NSF IBN 9982913 (ERD).

.294 Poster : Umami Taste

DISCRIMINATION BETWEEN THE TASTES OF SWEET STIMULI AND MONOSODIUM GLUTAMATE IN RATS

Heyer B.R.[1], Taylor-Burds C.C.[1], Mitzelfelt J.D.[1], Delay E.R.[1] *[1]Neuroscience Program, Regis University, Denver, Colorado*

Monosodium glutamate (MSG) is a naturally occurring amino acid found in protein-rich foods such as meat, vegetables, and dairy products, and elicits a taste called *umami*. Molecular and behavioral experiments (Chaudhari *et al.*,1996) indicated that MSG is transduced by taste-mGluR4 receptors. More recently, Nelson *et al.* (2002) reported that T1R1/T1R3, a broadly tuned amino acid receptor, responds to MSG but not to sweet stimuli. In contrast, T1R2/T1R3 receptors responded to a wide variety of natural sugars and artificial sweeteners but not to umami substances. However, conditioned taste aversion (CTA) experiments indicate that an aversion to MSG mixed with amiloride (a Na^+ channel blocker) generalizes strongly to natural sugars and artificial sweeteners and vice versa (Heyer *et al.*, AChemS Abs., 2002, Stapleton *et al.*, 1999), suggesting that the glutamate anion elicits a sweet taste. This was supported further when Stapleton *et al.* (2001) found that rats had difficulty discriminating between MSG with amiloride and sucrose below 100 mM. To further explore the "sweet" taste of MSG, shock-avoidance/water-reinforcement discrimination methods were used to test if rats could discriminate between MSG and glucose. Na^+ taste was reduced by adding amiloride to all solutions and equimolar NaCl to glucose. Rats reliably distinguished between a wide range of glucose concentrations and 10-150 mM concentrations of MSG (with amiloride). This suggests that the sweet taste of MSG is not as ubiquitous as CTA experiments suggest. We are conducting similar discrimination experiments with saccharine and MSG. Supported by NSF IBN 9982913 (ERD).

295 Poster : Umami Taste

GUSTATORY EVOKED MAGNETIC FIELDS AND PERCEPTUAL CHARACTERS OF UMAMI SUBSTANCE IMP

Saito S.[1], Kobayakawa T.[1], Gotow N.[1] *[1]Neuroscience research institute, National institute of advanced industrial science and technology, Tsukuba, Japan*

We suppose there must be different brain activities for different gustatory perception. The purpose of this study is to reveal different brain activities of umami perception by disodium 5' inosinate (IMP) from salty perception by NaCl. The gustatory evoked magnetic fields (GEMs) were measured by using 64 channels' whole-head SQUID MEG system and specially developed taste stimulator to measure evoked response. The perceptual characters, i.e. reaction time, perceived intensity, and taste quality were measured. 3 volunteers participated in GEMs and reaction time experiments using IMP and NaCl. 180 mM IMP and 300mM NaCl were presented 40 times by the stimulator, which supplied stimulus flow through a small area (8mm x 2.5mm) on Teflon tube. Participant reported a perceived intensity of 0 to 5.0 by using their fingers a few seconds after stimulation in each trial, and also reported taste quality after 40 trials. As the results, perceptual characters of IMP were different from NaCl as to reaction time, perceived intensity, and taste quality. The primary gustatory area (aera G) was estimated in both stimuli, but the first peak of GEMs for IMP showed less sharp tendency than that for NaCl. These results showed the different brain activities for IMP perception from NaCl perception. Since the sample size is still small, we shall have to conduct further experiments using many participants to strengthen these findings

296 Slide : Molecular Basis of Sweet Taste

COMPARATIVE ANALYSIS OF THE T1R TASTE RECEPTOR GENES IN PRIMATES

Li X.[1], Li W.[1], Mascioli K.J.[1], Bachmanov A.A.[1], Tordoff M.G.[1], Epple G.[1], Beauchamp G.K.[1], Reed D.R.[1] *[1]Monell Chemical Senses Center, Philadelphia, Pennsylvania*

Previous studies have shown differences in sweet taste perception among mammalian species, especially for nonsugar sweeteners. For instance, Prosimii (Prosimians) and Platyrrhini (New World primates) apparently do not taste aspartame as sweet, whereas Catarrhini (Old World monkeys, and apes) and humans do (Glaser et al., 1995). Because two members of the T1R taste receptor family, T1R2 and T1R3, were shown to function as a sweet taste receptor complex, we hypothesized that the sequence variation in these taste receptors may account for the species differences in taste responses to aspartame. To test this hypothesis, we have begun to examine sequences of the T1R2 and T1R3 genes (*TAS1R2* and *TAS1R3*) in 18 primate species (animals from 12 species taste the sweetness of aspartame and animals from the other 6 do not). Using PCR with primers based on human *TAS1R2* and *TAS1R3* sequences, we have obtained the entire or partial *TAS1R2/TAS1R3* coding sequences from these primate species. Our preliminary data show that at the amino acid level, there are eight sequence variant sites in T1R3 and nineteen variant sites in T1R2 between the aspartame-sensitive and insensitive groups. These sequence variants may affect the binding of aspartame to the T1R2/T1R3 receptor complex.

This work was supported by National Institutes of Health grants, R01DC00882 (GKB), R01DK55853 (DRR), and R01DC04188 (DRR).

297 Slide : Molecular Basis of Sweet Taste

T1R RECEPTORS ARE EXPRESSED BY TYPE II TASTE CELLS IN THE MOUSE

Finger T.E.[1], Anderson K.T.[1], Bartel D.[1], Yee C.L.[1] *[1]Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO*

The two families of cloned taste receptors T1Rs (for sweet-tasting substances) and T2Rs (for bitter-tasting substances) each are expressed by a subset of cells in taste buds in rodents. The T2Rs, but generally not T1Rs, are present in cells that also express gustducin, an obligate partner for detection of bitter compounds. Taste buds comprise three distinct morphological types of cells (Type I[dark], Type II [light] and Type III) which can be identified by distinct histochemical features. Previous studies have shown that gustducin (and therefore T2Rs) are expressed by a subset of Type II taste cells. We used combined in situ hybridization and immunocytochemistry to determine which type of taste cells express the T1R receptors, T1R1 and T1R2. We find that T1Rs co-exist in cells expressing PLC B2 (phospholipase C - beta 2), which is present in most Type II cells. T1Rs only occasionally co-exist in the subset of Type II cells expressing gustducin. T1Rs are not expressed in Type III taste cells which are identified by either serotonin-like or NCAM-like immunoreactivities. Therefore Type II taste cells ("light" cells) fall into two classes: those that express T1Rs, and those that express T2Rs and gustducin. Supported by NIH Grant PO1 DC00244

298 Slide : Molecular Basis of Sweet Taste

RELATIVE SENSITIVITY TO SUCROSE, GLUCOSE AND GLYCINE IN SELECTIVE INBRED MOUSE STRAINS.

Eylam S.[1], Spector A.C.[1] *[1]Dept. of Psychology and Ctr. for Smell and Taste, University of Florida, Gainesville, FL*

Recently, a family (T1R) of G-protein-coupled taste receptors forming heteromeric complexes that bind with some "sweet-tasting" ligands were identified. To provide a functional context in which to understand these molecular findings and to describe "sweetener" sensitivity in mouse strains that differ in sucrose preference, C57BL/6J (B6), SWR/J (SWR), 129P3/J (129) and DBA/2J (D2) mice previously tested with sucrose and D-phenylalanine (presented at AChemS XXIV) were further tested with glycine, a reportedly "sweet" amino acid, glucose, and sucrose. Water-restricted mice (n=9-12/strain) were trained in a specially designed gustometer to lick from one side spout in response to a taste stimulus sample and from another side spout in response to a water sample to receive a water reinforcer. As shown previously, sucrose threshold, arbitrarily defined as the concentration producing ½ asymptotic performance, differed across strains: B6 (.07M) ≈ SWR (.08M) < 129 (.15M) ≈ D2 mice (.18M). A similar pattern of strain differences was found for glucose (B6=.25M; SWR=.33M; 129=.58M; D2=.57M). The strain sensitivity pattern for glycine was distinct with a significant difference found only between B6 and 129 mice (B6=.04M; D2=.05M; SWR=.07M; 129=.11M). Correlations of thresholds collapsed across strains suggested that sucrose sensitivity is strongly related to that for glucose (r=.81), but is much less related to that for glycine (r=.42). These results suggest (but do not prove) that at the taste receptor level there is more similarity between sucrose and glucose than between glycine and either sugar at low concentrations. Supported by NIDCD R01-DC04574.

299 Slide : Molecular Basis of Sweet Taste

POLYMORPHISMS OF THE MURINE TAS1R3 GENE ASSORT WITH STRAIN DIFFERENCES IN OROSENSORY RESPONSIVENESS TO LOW BUT NOT HIGH CONCENTRATIONS OF SUCROSE

Glendinning J.[1], Joon Young M.[1], Lin I.[1] [1]*Biological Science, Barnard College, Columbia University, New York, New York*

Several recent reports have identified a family of "sweet" taste receptors in mammals, called the T1Rs. Two members of this family, T1R2 and T1R3, are thought to combine to function as a sweet receptor. One critical line of support for this work comes from behavioral studies of mouse strains with ploymorphisms of the gene (Tas1r3) that codes for T1R3. According to 48-hr preference tests, the Tas1r3 polymorphisms assort between mouse strains that differ in responsiveness to sweeteners (i.e., between taster and nontaster strains). Because the 48-hr preference test confounds orosensory and viscerosensory response mechanisms, we re-evaluated the mice with a brief-access testing paradigm that minimizes the contribution of viscerosensory response mechanisms. In so doing, we hoped to gain deeper insight into the orosensory consequences of allelic variation at Tas1r3. We used 3 strains of `tasters´ (C57BL/6, SWR, & FVB/N) and 4 strains of `nontasters´ (DBA/2, 129, BALB/c & C3H/HeJ). We found that the taster strains licked significantly more vigorously than the nontaster strains from low sucrose concentrations (i.e., < 0.2 M). In contrast, we found substantial overlap among taster and nontaster mouse strains in licking responsiveness to high sucrose concentrations (i.e., > 0.3 M). In fact, some nontaster strains licked as quickly as taster strains. Our results indicate that (a) allelic variation at Tas1r3 affects orosensory responsiveness to low but not high concentrations of sucrose; and (b) the genetic basis of strain differences in orosensory responsiveness to sweeteners may be more complex than previously suggested. Supported by NIH grant DC004475 (to JIG).

300 Slide : Molecular Basis of Sweet Taste

SWEETENER PREFERENCES OF 129.B6-SAC CONGENIC MICE

Theodorides M.L.[1], Li X.[1], Botchkarev V.V.[1], Beauchamp G.K.[1], Bachmanov A.A.[1] [1]*Monell Chemical Senses Center, Philadelphia, PA*

The mouse saccharin preference (*Sac*) locus is identical to a taste receptor gene, *Tas1r3*, which encodes a T1R3 protein. To assess ligand specificity of this receptor, we examined taste preferences of 129.B6-*Sac* congenic mice. These mice have been produced using serial backcrossing to introgress a *Tas1r3*-containing donor chromosomal fragment from the C57BL/6ByJ (B6) strain onto the genetic background of the 129P3/J (129) strain. Mice from the B6 and 129 strains have different alleles of the *Sac*/*Tas1r3* gene and differ in taste responses to several sweeteners. The assumption of this experiment was that if the *Tas1r3* genotype affects taste responses to a sweetener, then this sweetener acts as a ligand for the T1R3 receptor. Mice from the N8 or equivalent backcross generations were tested using 48-h two-bottle tests with concentration series of fourteen sweeteners. Congenic mice that had a copy of *Tas1r3* from the B6 strain had higher preferences for sucrose, glucose, maltose, fructose, saccharin, acesulfame, sucralose, SC45647, erythritol, D-phenylalanine, D-tryptophan and L-proline, compared with their littermates homozygous for the 129 allele of *Tas1r3*. Thus, allelic variation of *Tas1r3* affects behavioral taste responses to these sweeteners, suggesting they are T1R3 ligands. There were no differences between the two *Tas1r3* genotypes in preferences for glycine and L-alanine (or any of the control non-sweet solutions: quinine, citric acid, NaCl, IMP or MSG), suggesting that these two sweet-tasting amino acids may induce sweetness via a transduction mechanism not involving T1R3. Supported by NIH grant R01DC00882 (GKB).

301 Symposium : Presidential Symposium: Biology & Chemistry of Floral Scent

INTRODUCTION: THE BIOLOGY AND CHEMISTRY OF FLORAL SCENT

Hildebrand J.G.[1] [1]*ARL Division Of Neurobiology, University of Arizona, Tucson, Arizona*

Humans, and perhaps other animals, find floral scent pleasurable and make various uses of those natural odors. But why do flowers produce their delightful scents? Why are they chemically complex? How can we learn about their chemical composition? How do flowers make the constituent volatile compounds? What are the natural, "original" biological functions of floral scent? How do insects that are attracted by floral odors detect and process that chemical information? Finally, how and why do humans respond to the fragrances of flowers as we do? In this symposium, questions like these will be addressed by a natural-products chemist who is an expert in food, flavor and fragrance chemistry; a biologist who specializes in the genetics, biochemistry, and biological roles of floral production of volatile chemicals; a neurobiologist who explores the central processing of odors in the brains of honey bees that serve as floral pollinators; and an industrial scientist who is interested in behavioral and chemical aspects of chemosensory stimuli as they affect humans. Shakespeare reminded us that "That which we call a rose / By any other name would smell as sweet." But there is more to the story, and this symposium will enhance your appreciation floral odors and help make sense of those scents!

302 Symposium : Presidential Symposium: Biology & Chemistry of Floral Scent

FLORAL SCENT COMPOSITION - STRATEGIES FOR COMPONENT IDENTIFICATION

Bicchi C.[1] [1]*Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italy*

Determination of floral scent composition is fundamental for all further research work in this field. Analytical techniques have evolved greatly over the last ten years, allowing us to design new strategies to study floral scent composition and to investigate phenomena connected to the emission of volatiles from plants. This is due to several factors including: 1) the recent renew of interest in headspace sampling, producing a new approach known as High Concentration Capability Static Headspace Techniques (HCC-SHS e.g. HS-SPME, HSSE, HS-SPDE); 2) the possibility of dramatically increasing the speed of GC and GC/MS analysis, thanks both to fast and superfast GC and to fast mass spectrometry through time-of-flight MS systems (TOF-MS), which have shortened the analysis time to minutes or even seconds; and 3) the success of cyclodextrin derivatives as chiral selector for enantioselective GC, which have afforded the direct separation of the enantiomers of most racemates. Moreover, new analytical techniques aiming to establish direct relationships between sensory evaluation and chemical analysis (e.g. PTR-MS) have been developed; together with GC-MS, they can be used to correlate chemical to sensory analyses, or to follow almost on-line the volatiles emitted from living plants, e.g. to study the evolution of floral scent composition over time or the plant instantaneous reaction to a stressing situation. This lecture aims to present a short overview on the advancement of the analytical techniques and how they can influence the strategies and the approaches to adopt to study the volatile fraction emitted from flowers and the involved phenomena.

303 Symposium : Presidential Symposium: Biology & Chemistry of Floral Scent

DISTINGUISHING SIGNAL FROM NOISE IN COMPLEX FLORAL FRAGRANCES: A BIOLOGICAL PERSPECTIVE

Raguso R.A.[1] [1]*Biological Sciences, University of South Carolina, Columbia, SC*

Why are fragrances so complex? Flowers emit tens to hundreds of volatiles, whose blends are species-specific even among related plants sharing the same pollinator. Are all scent components necessary for plant fitness? From an evolutionary perspective, compounds that attract pollinators, repel enemies or otherwise contribute to plant defense constitute "signal". The remaining "noise" in fragrances might result from neutral genetic variation, "sloppy" biosynthetic enzymes or phylogenetic constraints. Most flowers use scent as an "honest signal" indicating pollen or nectar, but deceptive flowers dupe pollinators with odors that mimic sex pheromones, oviposition hosts (feces, carrion, fungi) or generalized food odors. Different components may attract pollinators from a distance, release feeding and other innate behaviors, or be learned as conditioning stimuli. Thus, diversity of behavioral function, biosynthetic and phylogenetic origins account for some of fragrances' complexity. Cluster analysis and multidimensional scaling help to visualize differences among floral scents in "multidimensional scent space". Human perception and traditional pollination biology predict that fragrances should form distinct clusters indicating pollinator "syndrome", but fragrances cluster only when "noise" components are subtracted from them. The most surprising pattern is a large cluster of plants with generalized pollination that share such broadly attractive, salient odors as linalool, ocimene, benzyl alcohol and 2-phenylethanol. Their ubiquity and prominence in floral scents suggest that fragrances are less complex than they seem.

304 Symposium : Presidential Symposium: Biology & Chemistry of Floral Scent

CODING FLORAL ODORS IN THE INSECT BRAIN

Galizia G.[1], Mustaparta H.[2], Sachse S.[1] [1]*Neurobiologie, Freie Universität Berlin, Berlin, Germany;* [2]*NTNU, Trondheim, Norway*

The insect analogue of the olfactory bulb is the antennal lobe (AL). Odors activate a combinatorial pattern of glomeruli, which can be measured using in-vivo calcium imaging. In many insect species, a 'macroglomerular complex' processes pheromone information. The remainder of the AL consists of 'ordinary' glomeruli, which process non-pheromonal odors. Floral volatiles are the most important odors for insects foraging on nectar or pollen but present particular challenges: they appear in plumes (with complex temporal patterns), consist of many substances (mixtures), and appear at varying concentrations. We have addressed these issues in moths and bees. When we gave the same stimulus with intervals ranging from every 60 sec to every 5 sec, some glomeruli responded to each stimulus, also at high frequencies, while others stopped responding with increasing frequency. Thus, an odor plume in a sequence leads to a different activity pattern from a solitary plume. The response to a mixture approximated the overlap of the components' responses. However, differences appear with increasing number of components. Mixture interactions can be modified by interfering with the inhibitory network of the AL. Increasing odor concentration led to stronger and more widespread responses, but some glomeruli were specifically inhibited. Thus, concentration invariance was not complete, maintaining information about concentration itself. We conclude that the AL network optimizes also information about temporal structure, mixtures and concentration. Therefore, the AL appears to be involved in encoding both odor quality and 'contextual' information.

305 Symposium : Presidential Symposium: Biology & Chemistry of Floral Scent

HUMAN RESPONSES TO FLORAL ODORS

Van Der Pers-King B.[1] [1]*sensory sciences, Quest International Nederland BV, Naarden, Netherlands*

In contrast to insects, humans have no life-or-death dependence on floral odors. Floral notes do contribute to our enjoyment of life, however. Throughout history flowers have been used to create a pleasant atmosphere, often by camouflaging a bad one. Floral notes form the basis of feminine perfumes. Classically floral notes in perfumery have been separated into six groups, two of which correspond directly to specific types of flowers (*florals* such as rose, violet, carnation and *white florals* such as muguet, jasmine, tuberose, orange blossom) whereas the other four have more generic names: *aldehydic, green, marine, fruity*. From a sensory point of view, this classification is rather empirical. It could be argued that fruits are more important to the human diet than flowers, thus fruity odors should be more easily identified than floral odors, or at least these two groups in the classification should be separable. This hypothesis, as well as the placement of notes such as green, which are common to both groups, was studied by correspondence analysis. Commercial perfumes representative of these floral classes were identified and linked to the marketing images used to sell them since the human response to floral odors is rather complex behavior.

306 Poster : Olfaction in Animal Behavior

CONCANAVALIN A INHIBITS DETECTION OF D-CARVONE WHILE WGA REDUCES DETECTION OF L-CARVONE

Deutsch S.[1], Apfelbach R.[1] [1]*Dept. of Zoology, University of Tuebingen, Tuebingen, Germany*

Lectins, carbohydrate-binding proteins, specifically bind to olfactory receptors via their sugar specific sites. In the present study we applied (by intranasal perfusion of the olfactory mucosa) the lectins Concanavalin A (Con A) or Wheat germ agglutinin (WGA) to the rat olfactory epithelium and tested the animal's ability to detect the carvone enantiomers (D and L optical isomers). For humans D-carvone has a caraway note and L-carvone a spearmint note. In our experiments odor detection tests were assessed in an air dilution olfactometer; animals were trained to discriminate low concentrations of D-carvone from clean air (group 1; n = 5) or to discriminate L-carvone from clean air (group 2; n = 5). Before lectin treatment rats reached a performance level of at least 90% correct responses (cr). Con A (2 mg/ml) reduced the D-carvone detection ability (58% cr) but had no effect on the L-carvone detection ability (90% cr). In contrast to this WGA (2 mg/ml) reduced detection of L-carvone (76% cr) yet no effect on the detection for D-carvone (95% cr) could be detected. Lectin-induced inhibition of carvone detection was reversible; the inhibitory effect for both lectins lasted for about 24 h. Our data provide strong evidence that the detection of enantiomers of carvone is mediated by different populations of sensory neurons. We thank Ram Research Ltd, London, for generous financial support.

307 Poster : Olfaction in Animal Behavior

REVERSIBLE REDUCTION OF ODOR DETECTION AFTER TROMETAMOL APPLICATION TO THE OLFACTORY EPITHELIUM OF RATS

Weiler E.[1], Heisig C.[2], Apfelbach R.[2] *[1]Neurophysiology, Ruhr-Universitaet, Bochum, Germany; [2]Zoology, University of Tuebingen, Tuebingen, Germany*

Trometamol (2-amino-2-hydroxymethyl-1,3-propanediol) is used in medicine e.g. to cure metabolic acidosis, as a gastrointestinal and diuretic drug, as a solvent of drugs used in aerosol containers, as well as in histological research as a buffering agent ("TRIS-buffer"). Preliminary experiments revealed that TRIS-buffer applied to the olfactory epithelium (OE) by intranasal perfusion of the olfactory mucosa reduces olfactory sensitivity. We, therefore, investigated this effect in greater detail and compared the trometamol effect with the effect caused by Ringer solution applied to the OE. Olfactory sensitivity was assessed in an air dilution olfactometer. Rats were trained to respond in an operant paradigm to low odor (ethyl acetate and n-octanal) concentrations. Prior to the perfusions with TRIS-buffer or Ringer solution of the OE animals reached performance levels of at least 90% correct responses (cr) independent of the odor offered. Treatment of the OE with Ringer solution revealed no effect on odor detection. TRIS-buffer reduced detection performance 30 min after treatment to 61.4 ± 5.6 % cr (EA) and 49.3 ± 5.3 % cr (n-octanal). Yet, the inhibitory effect is reversible: four hours after treatment detection performance returned to 90 ± 12.2 % cr (EA) and to 91 ± 10.2 % cr (n-octanal). Further studies will have to reveal the underlying mechanisms.

308 Poster : Olfaction in Animal Behavior

FETAL ODORS REMAIN IN THE MOTHER'S CIRCULATION AFTER HER PUPS ARE BORN

Yamazaki K.[1], Curran M.[1], Beauchamp G.K.[2] *[1]Monell Chemical Senses Center, Philadelphia, PA; [2]Monell Chemical Senses Center, Philadelphia, Pennsylvania*

MHC-determined odors, produced by fetuses, can be detected by other mice (Mus musculus). Theoretically, it should be possible to determine the MHC type of the sire based on the odortype of the pregnant female. Recent studies show that there is bi-directional cell traffic between mother and fetus during pregnancy: Some fetal cells colonize maternal tissue and remain there for considerable periods of time. We wondered whether fetal cells might provide the mother with odorants indicative of her offspring. To test this idea, inbred genetically-identical B6-H-2k females were divided into two groups, females in one group were mated with B6-H-2k males yielding kk fetuses, whereas females in the other group were mated with B6 males, yielding kb fetuses. Stud and female remained together until 14-15 days of gestation and were then separated. At birth, litters were permanently removed from the mothers. Urine of each post-partum female was collected overnight in a metabolic cage on days 1, 3, 5 and 7 after litter delivery and removal. We investigated whether urine from these genetically identical females would be discriminated by trained mice in the Y-maze previously extensively used for identifying H-2 odortypes in infant and adult mice. All six trained mice made these distinction at 1 and 3 days after litter delivery but did not discriminate at 5 or 7 days after litter delivery. Thus, fetal odor remain in the mother´s circulation until at least post-partum day 3; other bioassay procedures may show a longer persistence. To our knowledge, these are the first data indicating fetal effects on post-partum odor. Supported by NSF 0112528

309 Poster : Olfaction in Animal Behavior

DISCRIMINATION OF AMINO ACID MULTIMIXTURES IN CATFISH

Valentincic T.[1], Kralj S.[1], Zgonik V.[1] *[1]Biology, University of Ljubljana, Ljubljana, Slovenia*

We investigated in black bullhead catfish (*Ictalurus melas*) olfactory discrimination of multimixtures composed of 4-7 and 10-13 equally effective amino acids. Our previous study indicated that binary and ternary mixtures of amino acids were initially detected as their most stimulatory components; the presence of minor components in the mixture was perceived only after 5-10 discrimination training trials. The catfish were conditioned to the amino acids mixtures composed of either seven or thirteen equally effective amino acids. Equal effectiveness of the tested amino acids was achieved by adjusting the concentrations of the components to provide equal EOG amplitudes. Mixtures of four components were discriminated immediately from the conditioned mixture of seven amino acids. Five to eight discrimination training trials were necessary to enable the discrimination of five to six component mixtures from the conditioned seven component mixture. Black bullhead catfish discriminated the thirteen amino acid mixture from its ten and eleven component counterparts; however, irrespective of the number of the discrimination training trials, the catfish were unable to discriminate the thirteen component conditioned mixture from its twelve component counterpart. In conclusion, multimixture discrimination improves with the number of discrimination training trials; however, catfish cannot learn to discriminate the thirteen component conditioned mixture from the mixtures different by one component. Funded by Ministry of Education and Science Grant P0-0509-0487 to Tine Valentincic.

310 Poster : Olfaction in Animal Behavior

CANINE OLFACTORY THRESHOLDS TO N-AMYL ACETATE MEASURED NATURALISTICALLY

Walker D.B.[1], Cavnar P.J.[1], Taylor J.L.[1], Pickel D.H.[2], Suarez J.C.[1], Hall S.B.[1], Walker J.C.[1] *[1]Sensory Research Institute, Florida State University, Tallahassee, FL; [2]Von Pickel K-9, Inc., Tallahassee, FL*

We have developed (Pickel et al., 2002) a procedure that fuses the key elements of animal odor psychophysics with those present when dogs are working with their handlers to solve "real world" problems of odor identification and localization. Each dog is deprived of neither food nor water, housed with its trainer-handler and works collaboratively with this individual to determine which of five Teflon boxes contains the target odorant of n-amyl acetate (nAA). In two dogs (Rottweiler, standard schnauzer) trained in this way, nAA concentrations were systematically lowered over the course of several weeks, in blocks of three 9-trial sessions, until chance performance was seen. Data for each concentration were expressed in terms of a logistic regression equation relating concentration to the binomial probability that the observed performance (or better) would be seen by chance alone. That concentration corresponding to a probability of 0.05 was defined as threshold. In this admittedly small sample, the values we obtained (1.9 and 1.14 parts per trillion) are 40- to 17,000-fold lower than the range of thresholds reported by Krestel et al. (1984) in their conditioned suppression study of beagle sensitivity to nAA. Thus, it appears that there are significant advantages to our approach, though the reasons for differences in results are unclear. The "find the target" aspect of this new method makes it readily applicable to odor processing tasks much more complex than detection of single compounds.

311 Poster : Olfaction in Animal Behavior

ADENOVIRAL VECTOR-MEDIATED RESCUE OF THE OMP-NULL BEHAVIORAL PHENOTYPE: ENHANCEMENT OF ODORANT SENSITIVITY

Youngentob S.L.[1], Pyrski M.[2], Margolis F.L.[3] *[1]Neuroscience and Physiology, SUNY - Upstate Medical University, Syracuse, NY; [2]Anatomy & Neurobiology, University of Maryland at Baltimore; [3]Medicine, University of Maryland at Baltimore*

OMP-null mice demonstrate a constellation of neurophysiological and behavioral defects that suggest OMP is critically involved in odor processing. The current study examined adenoviral vector-mediated gene replacement on the re-acquisition of behavioral function. Thirty-three null mice (11 OMP-null/OMP-EGFP-AdV, 11 OMP-null/no virus control and 11 OMP-null/EGFP AdV control) were trained, appropriately inoculated and then tested for their sensitivity to the odorant propanol. Mice receiving no viral inoculation or inoculation with an EGFP virus were included to control for non-specific negative effects of infection and/or EGFP toxicity. Following training on a go-no-go paradigm, viral animals were inoculated and then rested for two days to allow for appropriate protein expression. On day three a threshold estimate for the odorant propanol was determined. The results (mean log concentration +/- SE) were: OMP + EGFP, - 4.15 +/- 0.15; no virus, -3.91 +/- 0.20; and EGFP only, -3.55 +/- 0.22. Animals receiving OMP + EGFP were more sensitive than were no-virus controls, and non-inoculated controls were more sensitive then animals receiving only EGFP. Animals receiving the OMP gene were more sensitive than those receiving EGFP by a factor of 3.98 ($p=0.02$) and the improvement was uniform across all concentrations ($p=0.04$). These data suggest: (1) there is a nonspecific effect that masks the full impact of vector-mediated rescue of OMP; and (2) replacing OMP improved sensory function confirming and extending upon previous suggestions that OMP plays a direct role in the odor detection/signal transduction cascade. NIDCD Grant - DC03904

312 Poster : Olfaction in Animal Behavior

A NEW APPROACH TO COMMUNICATION IN BIRDS: STRUCTURE AND FUNCTION OF NASAL ANATOMY

Yeh J.[1], Launer K.[1], Hagelin J.C.[2] *[1]University of Connecticut, Storrs, CT; [2]Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT*

It is widely believed that birds have no sense of smell. Yet, every bird studied has a functional olfactory system. Such misconceptions have led to little study of chemical communication in birds. The auklets, a group of Alaskan seabirds, provides a unique 5-species system for integrating studies of nasal structure and odor signals. One member of this group, the Crested Auklet (Aethia cristatella), exhibits a citrus plumage odor that appears to be linked with a well-studied courtship behavior. Thus, Crested Auklets may use chemical signals, while other species may not. One might predict that the Crested Auklet differs from its unscented relatives in displaying a more complex machinery devoted to olfaction. Using histological techniques, we calculated the total surface area of olfactory epithelium for Crested Auklets and four related species. The total olfactory epithelium averaged 65.4 +/- 15.1 mm2 for Crested Auklets and 57.1 +/- 16.9 mm2 for other species. A comparison of fledgling vs. adult birds revealed marked differences in olfactory epithelium. Fledglings, which leave the nest and remain at sea for several years, exhibited very little fully developed olfactory epithelium. Fledgling tissue consisted of a thick basal layer with some receptor cells migrating to the surface. Our work suggests that scented and unscented auklets do not exhibit marked differences in the total amount of olfactory epithelium. Fledglings of all species apparently undergo more extensive olfactory development at sea.

313 Poster : Olfaction in Animal Behavior

TIMING OF *MANDUCA SEXTA* BEHAVIORAL AND PHYSIOLOGICAL TRAITS DURING WANDERING.

Kobres R.P.[1], Fernandez K., Vogt R.G. *[1]Department Of Biology, University of South Carolina, Columbia, SC*

Manduca sexta larvae are eating machines from the time they hatch from the egg to a pre-pupation stage called wandering. When an animal wanders, it ceases eating and begins an approximate 4.5 day period of metamorphosis. During this time, the pre-pupa structures form beneath the larval skin. This transition into wandering has been defined by several behavioral and physiological criteria: cessation of feeding, appearance of the heartbeat as a strong dorsal dark blue line, and burrowing. Unlike wandering, ecdysis occurs nearly instantly and provides a concrete time point for tissue studies. A definition for the transition through wandering was needed as a foundation for precise temporal studies of tissues between wandering and pupation. Using time-lapse video equipment and infra-red lighting, the transition from late 5th instar to a wandering animal has been documented in relation to ecdysis. Behavioral changes along with weight data were recorded. Preliminary data reveal that in short day cycle animals, several behavioral motifs occur. During the night hours every animal will typically cease feeding minutes after the lights go out. They move to a resting location, followed by an intriguing period of curling. Finally each drops off the elevated platform. These behaviors may be under circadian control, and/or mediated by the dramatic hormonal changes that occur at this time.

314 Poster : Animal Behavior: Taste & Feeding

MURINE TASTE PREFERENCE TESTS: WHY ONLY TWO BOTTLES?

Tordoff M.G.[1], Bachmanov A.A.[1] *[1]Monell Chemical Senses Center, Philadelphia, Pennsylvania*

Two-bottle tests have been used extensively to measure the preference for taste and nutrient solutions but there has been little work with tests involving more than two bottles. Here, we compare the results obtained in two-bottle tests with those obtained in three- and six-bottle tests. In Experiment 1, we measured the preferences for 2 mM saccharin, 50 mM citric acid, 0.3 mM quinine hydrochloride and 75 mM NaCl displayed by 129X1/SvJ (129) and C57BL/6J (B6) mice. When mice received three bottles, two providing a taste solution and one providing water, they drank more of the taste solution than when they received a standard two-bottle test, or two spouts providing water and one providing taste solution. The three-bottle tests also revealed the left spout side preferences of the 129 strain and were generally better at distinguishing between the 129 and B6 strains (i.e., were more sensitive) than were two-bottle tests. In Experiment 2, we measured intakes and preferences in tests with 6 bottles, with 1, 2, 3, 4, or 5 containing 75 mM NaCl and the rest containing water. NaCl preferences were monotonically related to the number of NaCl spouts available. A follow-up experiment found similar results whether the index of ingestion was volume intakes or licks. This argues that spillage cannot account for the effect of spout number on taste solution intake. Together, the results suggest that (a) the number of bottles of taste solution and water has a profound influence on taste solution intake and preference, and (b) three-bottle tests may be more sensitive than two-bottle tests in many circumstances. Funded by NIH AA-12715.

315 Poster : Animal Behavior: Taste & Feeding

PROP AND PTC TASTE SENSITIVITY ARE DISTINCT PHENOTYPES IN MICE

Nelson T.M.[1], Munger S.D.[1], Boughter J.D.[2] [1]*Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD;* [2]*Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN*

Different mammalian species exhibit unique taste sensitivities that may reflect specific genetic variations. In humans, taste sensitivities to the chemically similar, bitter-tasting compounds phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) are heritable and strongly correlated, suggesting a common genetic basis. Here we report that PROP and PTC taste sensitivities vary independently between two inbred strains of mice. In brief–access taste tests, C3HeB/FeJ (C3) mice are more sensitive to PTC than are SWR/J (SW) mice, while SW mice are more sensitive to PROP than are C3 mice. However, SW mice are more sensitive to both compounds in 48h intake tests. This discrepancy is explained by the observation that SW mice consumed taste solutions at a greater rate during the intake test than did C3 mice. Therefore, PTC avoidance correlated with the amount of PTC consumed in the intake tests, rather than the concentration of PTC tested. These findings suggest that post-ingestive factors play a significant role in PTC avoidance during intake tests and highlight an important advantage of brief-access tests over intake tests in resolving the gustatory and post-ingestive contributions to taste-related behaviors. Most strikingly, these results indicate that in mice, unlike in humans, PTC and PROP taste sensitivities vary independently, thereby suggesting a subtle functional diversity of bitter-taste mechanisms across mammalian species. Support: NIDCD grants DC005786 (S.D.M.) and DC004935 (J.D.B.).

316 Poster : Animal Behavior: Taste & Feeding

CETYLPYRIDINIUM CHLORIDE'S AVERSIVE TASTE TO RATS

Hallagan L.[1], Saperstein S.[1], St. John S.J.[1] [1]*Psychology, Reed College, Portland, Oregon*

Cetylpiridinium chloride (CPC) is a compound of considerable theoretical and practical interest in gustation because of its NaCl-enhancing (e.g., at 0.25 mM) and NaCl-inhibiting (2 mM) effects in rat chorda tympani (CT) nerve. Most interestingly, the CPC-sensitive portion of the NaCl response appears to be independent of the amiloride-sensitive portion (as assessed at the whole-nerve level), making CPC a prime candidate for behavioral and single-unit electrophysiological studies analyzing taste quality coding of salts. As a precursor to those studies, we assessed whether CPC itself has a taste at relevant concentrations. Because the glossopharyngeal (GL) nerve does not appear to be necessary for a variety of tasks involving the detection and discrimination of NaCl, we also assessed the taste properties of CPC after GL, CT, and combined GL + CT transection. Water-deprived rats were trained to lick in an automated apparatus (MS-160) that provided access to distilled water and CPC (0.03, 0.1, 0.3, 1, 3, and 10 mM). The number of licks during 8 s trials (counted from the first lick) were the primary data. Rats were tested in 4 sessions (up to 8 trials/stimulus/session) prior to surgery (n=7 or 8/group) and in 4 identical sessions after surgery. Lick rates to CPC monotonically decreased with concentration, indicating it is aversive to rats. Surprisingly, neither GL nor CT transection substantially reduced avoidance, but the combined transection did (strongly suggesting the avoidance has an orosensory, rather than olfactory, basis). Unlike amiloride, which is tasteless to rats, investigators assessing the behavioral or electrophysiological effects of CPC on salt taste must therefore be aware of CPC's orosensory effects.

317 Poster : Animal Behavior: Taste & Feeding

DETERMINATION OF NACL TASTE THRESHOLDS FOR DEVELOPMENTALLY NACL-RESTRICTED RATS

Thaw A.K.[1], Mann E.C.[1], Crooms S.[1], Hill D.L.[2] [1]*Psychology, Millsaps College, Jackson, MS;* [2]*Psychology, University of Virginia, Charlottesville, Virginia*

The effect of sodium restriction on the taste system of rats has been extensively studied. Electrophysiological data indicate decreased sensitivity of whole nerve responses to NaCl solutions due to inactive amiloride sensitive sodium channels. Whether this physiological effect represents a decrease in taste sensitivity has not been examined behaviorally. The present study compared taste thresholds of control and developmentally NaCl-restricted rats to determine if early nutritional challenges lead to decreases in taste sensitivity. Using a conditioned suppression procedure, mildly food deprived rats (90% body weight) were trained to drink water from 8 individually presented sipper tubes to receive food reinforcements. Tubes were presented for 10 seconds and total licks were recorded. Once rats consistently licked all tubes, sucrose solutions were placed in half of the tubes. Rats received a mild foot shock if they licked sucrose solutions. Thus, rats began licking water tubes only and suppressing licks on the sucrose tubes. On the final day of testing sucrose solutions were replaced with sub-threshold and suprathreshold NaCl solutions. The rats continued to lick the water tubes and tubes in which they could not detect NaCl, while suppressing licking on NaCl solutions they could detect, allowing accurate thresholds to be obtained. Results indicate normal NaCl thresholds for control rats, but lower thresholds for NaCl-restricted rats. Thus, even though electrophysiological data show decreased nerve responses to NaCl solutions, developmentally NaCl-restricted rats do not display a decrease in taste sensitivity.

318 Poster : Animal Behavior: Taste & Feeding

GUSTATORY DETECTION OF A FREE FATTY ACID, LINOLEIC ACID, BY RATS.

McCormack D.N.[1], Herzog P.[1], Webster K.L.[1], Pittman D.W.[1] [1]*Psychology, Wofford College, Spartanburg, SC*

It has been established that the addition of corn oil to a tastant will increase the consumption of that tastant by rats. As the effects of post-ingestive signaling were minimized, it was suggested that this increase in consumption was based on orosensory information. Furthermore, it has been shown that rats will generalize a conditioned taste aversion formed initially to corn oil to a free fatty acid component of corn oil, linoleic acid, and vice versa (Smith et al, Physiol. Behav. 69:135, 2000). The current study characterizes the detection threshold for linoleic acid in rats through a conditioned taste aversion paradigm. Following a single pairing of a LiCl injection with consumption of linoleic acid at a concentration ≥66 μM, rats avoided future consumption of linoleic acid as compared to controls; however, the pairing of a LiCl injection with consumption of 44 μM linoleic acid did not produce a conditioned taste aversion. Assuming that orosensory information is the basis for avoiding subsequent linoleic acid consumption following a conditioned taste aversion, we sought to identify the necessary gustatory neural pathways. Bilateral transection of the chorda tympani nerve eliminated the formation of a conditioned taste aversion to 88 μM linoleic acid. These data suggest that rats can detect the presence of linoleic acid (≥66 μM) in the oral cavity and that the chorda tympani nerve transmits necessary neural information for the formation of a conditioned taste aversion to linoleic acid. Supported in part by a grant from the South Carolina Independent Colleges & Universities.

319 Poster : Animal Behavior: Taste & Feeding

THRESHOLD FOR THE DETECTION OF ETHANOL SOLUTIONS BY THE RAT

Smith J.[1], Denbleyker M.[2], Ferrence K.[2], Smith P.[2] [1]*Florida State University, Tallahassee, Florida;* [2]*Psychology Department, Florida State University, Tallahassee, FL*

Smith et al have shown that when a rat is given an injection of LiCl following a taste of linoleic acid that a subsequent aversion to the fatty acid solution is conditioned. The linoleic acid was mixed with one ml of ethanol in order to get it into solution. This one ml of solution was dissolved in four liters of distilled water, giving an ethanol concentration of .025%. The question arises as to the sensitivity of the rat to very low concentrations of the ethanol alone. Prior attempts to measure ethanol thresholds involved two-bottle preference tests with concentrations of ethanol only as low as 0.1%. Recognizing that detection of ethanol by the rat could be made with either taste or smell mediation, we used a conditioned flavor aversion technique to measure the threshold for detection of ethanol. We found that by pairing .025% ethanol solution with an injection of LiCl, we could condition a flavor aversion to this concentration. Following this conditioning, we lowered the concentration of ethanol in subsequent daily preference tests by small increments over several days of testing to a final concentration of .0025%. Most of the rats could detect concentrations of .005%, but not .0025%, resulting in a detection threshold that is almost two orders of magnitude lower than that previously reported. In a subsequent experiment, we observed that rats with olfactory bulb ablations could not detect ethanol concentrations at these low levels, indicating that the threshold was one of olfaction and not taste. Threshold data will be presented.

320 Poster : Animal Behavior: Taste & Feeding

LINOLEIC ACID ALTERS LICKING RESPONSES TO SWEET, SOUR, AND SALT TASTANTS IN RATS.

Herzog P.[1], McCormack D.N.[1], Webster K.L.[1], Pittman D.W.[1] [1]*Psychology, Wofford College, Spartanburg, SC*

Previous research has shown that linoleic acid, a free fatty acid, inhibits delayed-rectifying K+ channels in isolated rat taste receptor cells with a net effect of prolonged depolarization in response to taste stimuli (Gilbertson et al., Am. J. Physiol. 272(41):C1203, 1997). This experiment examined the licking behavior of rats (n=8) when linoleic acid was added to prototypical tastant solutions. The licking behavior to concentrations of sucrose (15-250mM), NaCl (30-1000mM), citric acid (1.5-60mM), and QHCl (0.75-30mM) presented as 20s trials in a Davis Rig apparatus was examined with and without the addition of 88 μM linoleic acid. Linoleic acid increased the licking responses to naturally reinforcing sucrose concentrations ≥62 mM in non-deprived rats. NaCl, citric acid, and QHCl are not naturally reinforcing stimuli and required 23hr water deprivation to elicit licking responses to all concentrations. Linoleic acid reduced the licking response across all NaCl concentrations except 1 M NaCl. Linoleic acid produced an increase in the licking responses to citric acid concentrations ≤30 mM. There was no effect of linoleic acid on the licking responses to QHCl concentrations. Rats responded to sucrose and NaCl as if the concentrations were more intense when linoleic acid was present. These data support the theory that linoleic acid may prolong the depolarization of taste receptor cells in response to taste stimuli through inhibition of basolateral K+ channels. The effect of linoleic acid on citric acid may reflect a modulation of K+ channels involved in sour taste transduction.

321 Poster : Animal Behavior: Taste & Feeding

THE RELATIVE STRENGTH OF THERMAL CUES IN SHORT-TERM FEEDING BEHAVIOR

Smith P.L.[1], Smith J.C.[1] [1]*Department of Psychology, Florida State University, Tallahassee, Florida*

Our laboratory has previously shown that thermal cues are effective stimuli in preference and avoidance behavior in the rat. In the following series of experiments that used a conditioned taste aversion paradigm, the relative strength of thermal cues in short-term feeding tests was studied. Experiment 1 looked at the minimal difference in temperature that rats can discriminate. Rats were trained to drink water at room temperature, after which each animal was given 10-minute access to 10°C water followed by either a saline or LiCl injection. Post-conditioning consisted of 2-choice tests between the CS and water at a different temperature that ranged between 13°C and 25°C on subsequent tests. The data show that LiCl-injected rats not only showed an aversion to the CS, but these animals clearly distinguished between a narrow range of temperature (as low as 6°C). Experiment 2 looked at the relative strengths of taste and thermal cues when both were present in the CS as a mixture. Rats were given 10 minute-access to 0.125% saccharin at 10°C followed by a saline or LiCl injection. Subsequent post-conditioning tests revealed that LiCl-injected rats avoided any substance that possessed at least one of cues from the original mixture. In Experiment 3, the relative strengths of these cues were further studied by measuring the extinction rates of aversions to a novel thermal cue, a novel taste cue, or a novel mixture of taste and temperature cues. These data suggest that thermal input is a relatively effective stimulus, but its interaction with taste input makes it an even more effective stimulus in feeding behavior.

322 Poster : Animal Behavior: Taste & Feeding

BEHAVIORAL AND IMMUNOHISTOCHEMICAL STUDIES FOR THE CONDITIONED TEMPERATURE AVERSION.

Sako N.[1], Ohashi R.[1], Sakai N.[2], Sugimura T.[1] [1]*Oral physiology, Asahi University, Motosu, Gifu, Japan;* [2]*Neuroscience Research Institute, Japan Society for Promoting Science (AIST), Tsukuba, Ibaraki, Japan*

It has been known that taste has the ability as the conditioned stimulus (CS) for the conditioned food aversion. In the present study, to examine whether temperature of the drinking water also has the ability as CS or not, we conducted behavioral and immunohistochemical experiments in Wistar rats. Results were as follows; (1) When the rats were aversive-conditioned to 5 or 40 °C distilled water (dw) by intraperitoneal injection of 0.15M LiCl (2% bw), they could acquire the conditioning to CS, but they did not avoid any taste stimuli including CS temperature. (2) When the rats were aversive-conditioned to 5 or 40 °C 0.1M sucrose, they generalized sucrose with all tested temperature. (3) The rats with bilateral lesions of amygdala could not acquire the conditioning to the temperature. (4) After conditioning to 5 or 40 °C dw, the expression of c-*fos* was shown in amygdala of the rats which drunk CS. These results suggest that rats have the ability of acquisition of the conditioned temperature aversion and amygdala plays an important role to acquire this conditioning.

323 Poster : Animal Behavior: Taste & Feeding

SALIVARY GURMARIN-BINDING PROTEINS INDUCED BY GYMNEMA-CONTAINING DIET IN RATS

Yamada A.[1], Katsukawa H.[2], Sugita D.[3], Nakata M.[4], Ninomiya Y.[5]
[1]Sect. Oral Neurosci.,and Pediatric Dentistry, Grad. Sch. Dental Sci., Kyushu University, Fukuoka, Japan; [2]Dept. Oral. Physiol., Dent., Asahi Univ., Hozumi, Japan; [3]Central Laboratory, Lotte Co., LTD., Saitama, Japan; [4]Sect. Pediatric Dentistry, Grad. Sch. Dental Sci., Kyushu University, Fukuoka, Japan; [5]Sect. Oral Neurosci., Grad. Sch. Dental Sci., Kyushu University, Fukuoka, Japan

A plant of *Gymnema sylvestre* is known to contain inhibitors for sweet taste responses in human (gymnemic acid) and rodents (gurmarin: Gur). Our previous studies demonstrated that when rat fed the gymnema diet (GD), behavioral preference for sucrose transiently decreased, but it subsequently returned to the control level. We found that some proteins in the submandibular saliva of rats fed the GD that interact with Gur. The present study further examined possible induction of salivary proteins and concomitant changes in behavioral preference for sweet substances by the GD. Preferences for 1 mM saccharin and 10 mM D-phenylalanine changed with similar time course after the GD to those for sucrose observed in the previous study. The analyses using electrophoresis indicated that some protein components, especially with molecular weights of about 20, 40 and 150 kDa increased by feeding with the GD. These components decreased to the control levels by bilateral glossopharyngeal denervation. The components with more than 100 kDa showed strongest inhibition of immunoreaction between Gur and anti-Gur antiserum. These results suggest that GD induced the Gur-binding proteins through chemical signals arising from the glossopharyngeal nerve in the rat submandibular saliva, which may inhibit the sweet-suppressive effect of Gur and lead to the restoration of sweet taste preference.

324 Poster : Animal Behavior: Taste & Feeding

EFFECTS OF ASTRINGANTS, TASTANTS, AND FLAVORANTS ON ORAL TEXTURE.

De Wijk R.A.[1], Prinz J.F.[2], Engelen L.[2], Janssen A.M.[3] *[1]ATO-DLO, Wageningen, Netherlands; [2]Utrecht Medical Center, Utrecht, Netherlands; [3]Wageningen Center for Food Sciences, Wageningen, Netherlands*

Interactions between oral texture and tastants, flavorants, and astringent substances were explored psychophysically, physico-chemically, and physiologically in a series of studies. The first study, where selected tastants and flavorants were added to custard desserts, demonstrated that diacetyl, benzaldehyde, and caffeine affected both fat-related sensations (rough, dry-mealy, fatty, and creamy) as well as viscosity-related ones (thickness and melting). These added substances also elicited trigeminal activation. The second study demonstrated significant concentration effects of added diacetyl and vanillin. The third study demonstrated that the benzaldehyde effect found in the first study disappeared when nose-clips were applied, i.e., the flavor effect on oral texture sensations seemed to be primarily mediated by nasal olfaction and/or irritation. This was confirmed by the results of the fourth study which showed no effect of a tastant (aspartane) on oral texture. Physico-chemical and physiological measurements indicated that added substances may affect perception of the structure of the desserts, either directly via physico-chemical interactions with starch or indirectly via a change in salivary viscosity and production. Previously, reduced salivary viscosity by precipitation of Proline-Rich-Proteins resulting in increased friction between oral tissues was hypothesized to be the (only) basis for astringency. However, the present studies indicated that 1) not all astringent substances produce salivary precipitation, 2) salivary viscosities for some astringents indeed decrease (tannic acid) but those for others actually increase (alum), 3) reduced salivary viscosities do not necessarily result in increased friction.

325 Poster : Animal Behavior: Taste & Feeding

THE IMPACT OF ETHANOL AND TOBACCO SMOKE ON INTRANASAL EPITHELIUM IN THE RAT

Vent J.[1], Irwin S.[1], Haynatzki G.[2], Gentry-Nielsen M.J.[3], Leopold D.A.[1], Hallworth R.[4] *[1]Otolaryngology, University of Nebraska Medical Center, Omaha, NE; [2]Department of Medicine, Creighton University, Omaha, NE; [3]Department of Medical Microbiology and Immunology, Creighton University, Omaha, NE; [4]Department of Biomedical Sciences, Creighton University, Omaha, NE*

Investigations have shown the influence of ethanol and tobacco smoke on olfaction. Analysis of ethanol and tobacco smoke-induced histopathologic mucosal changes in the upper respiratory tract may provide important insight into the pathophysiology of secondary olfactory dysfunction. 24 male Sprague- Dawley laboratory rats were randomly divided into two groups: group1 was exposed to tobacco smoke, group2 had no such exposure. After 6 weeks both groups were subdivided: 6 rats in each group were gradually switched to an isocaloric diet with 36% of the carbohydrate calories replaced by ethanol for 5 weeks. Epithelium was classified as olfactory, respiratory or unciliated epithelium by a trained microscopist, who was blind to the treatment status of the animals. Compared to controls, histopathologic analysis of nasal mucosa in exposed rats revealed a decrease in the amount of olfactory epithelium and loss of cilia, especially in the rats exposed to both ethanol and tobacco smoke. The histologic changes noted after ethanol and tobacco smoke exposure could explain the decreased olfactory ability seen in patients who excessively use these products. This study was supported by NIH grant AA13434 "Tobacco Smoke and Ethanol Induced Defects in Pneumonia Defense" and the Department of Otolaryngology-U.N.M.C.

326 Poster : Animal Behavior: Taste & Feeding

TASTE PERCEPTION IN MICE BEARING A HUMAN ORAL SQUAMOUS CELL CARCINOMA

Lee J.[1], Cha M.[2], Choi S.[2], Moon Y.[3], Jahng J.[2] *[1]Oral & Maxillofacial Surgery, Seoul National Univ. Coll. Dentistry, Seoul, South Korea; [2]Pharmacology, Yonsei University College of Medicine, Seoul, South Korea; [3]Biology, Catholic Univ. Med. Coll., Seoul, South Korea*

We previously showed that human oral squamous cell carcinoma (HOSC) induces anorexia in mice as the growth of tumor mass, and that NPY expression in the arcuate nucleus of tumor bearing mice was not increased in spite of persistent weight loss. Anorexic syndrome developed in cancer patients includes not only the loss of appetite and increased satiety, but also the disturbances in taste and smell. In this study, we performed the preference test for a sweet solution (G+S solution; 0.2% saccharine, 50% glucose) in tumor mice with two bottle test paradigm. Immortalized KB cell line originated from HOSC was subcutaneously inoculated in the back of nude mice at 8 weeks of age. Body weight, food and water intake was recorded every morning. Adipsia was developed as the tumor mass becomes obviously detected. When the tumor/body weight ratio reached 9-10%, mice were trained with two bottle drinking (water and empty bottles changed their positions every 24 h) for 5 days, then G+S was given instead of the empty bottle for 48 h with one change of the bottle position at 24 h. A slight but significant increase in G+S intake was detected in tumor mice, compared to non-tumor mice. Interestingly, water intake of the tumor mice which previously showed adipsia did not differ from the non-tumor control. Chow intake was decreased in both groups during the test days. These results suggest that sweet taste preference is altered in the tumor mice, and that this alteration may not be secondary to the reduction in food intake. Expression of a taste receptor gene, T1R and activation of a transcription factor, CREB were examined in the taste receptor cells to find a molecular mechanism of alterations in taste perception. Supported by KISTEP(JWJ).

327 Slide : Development of Chemoreceptor Cells

NEUROTROPHINS ALTER SODIUM CHANNEL PROPERTIES OF EMBRYONIC TRIGEMINAL AND GENICULATE NEURONS.

Grigaliunas A.[1], Mistretta C.[2], Maccallum D.K.[3], Bradley R.[2] *[1]Kaunas Medical University, Kaunas, LT; [2]School of Dentistry, University of Michigan, Ann Arbor, MI; [3]Medical School, University of Michigan, Ann Arbor, MI*

Neurons of trigeminal (tg) and geniculate (gg) ganglia innervate adjacent but distinct areas in fungiform papillae, where neurotrophins are differentially distributed. To study neurotrophin effects on action potential properties of embryonic neurons, tg and gg were dissected from rats at gestational day 13 (E13) or E16 and cultured. Ganglia were maintained with 10 ng/ml nerve growth factor (NGF) for tg or brain - derived growth factor (BDNF) for gg. Electrophysiological properties were recorded with whole cell current clamp. Action potentials of all E13 and E16 tg neurons were resistant to the sodium channel blocker tetrodotoxin (TTX), indicating presence of TTX-resistant (TTX-R) sodium channels in the tg. In contrast, all gg neuron action potentials were sensitive to TTX (TTX-S). However, when the tg was maintained in culture with NGF in combination with an increasing proportion of BDNF, the percentage of neurons with TTX-R action potentials decreased. For example, at E13, 100% of tg neurons had TTX-R action potentials in culture with NGF alone, but only 25% had TTX-R action potentials when 10 ng/ml BDNF was combined with 10ng/ml NGF, and 0% had TTX-R action potentials with BDNF alone. Gg neurons generated TTX-S action potentials, regardless of neurotrophin condition. Results demonstrate that tg and gg neurons have different sodium channel properties, and suggest that TTX-R channels in tg but not gg neurons are regulated by specific neurotrophins. Supported by NIDCD Grant DC00456 to CM.

328 Slide : Development of Chemoreceptor Cells

EACH SENSORY NERVE ARISING FROM THE GENICULATE GANGLION (GG) EXPRESSES A UNIQUE FINGERPRINT OF NEUROTROPHIN RECEPTOR GENES

Farbman A.I.[1], Sollars S.I.[2], Guagliardo N.[3], Hill D.L.[4] *[1]Neurobiology and Physiology, Northwestern University, Evanston, Illinois; [2]Psychology, University of Nebraska, Omaha, Omaha, Nebraska; [3]University of Virginia, Charlottesville, Virginia; [4]Psychology, University of Virginia, Charlottesville, Virginia*

The rat GG gives rise to 3 peripheral nerve branches, the chorda tympani (CT), the greater superficial petrosal (GSP) and a posterior auricular cutaneous nerve. The CT innervates 2 fields of taste buds, those in fungiform papillae and those in anterior foliate papillae. The GSP innervates taste buds in 2 other target fields, the incisive papilla and soft palate. The posterior auricular nerve innervates the skin of the external ear. To examine the pattern of neurotrophin receptor gene expression for each nerve each of the 5 target fields was injected with the markers, biotinylated dextran or fluoro-gold, both of which are retrogradely transported from the axon terminals to the neuronal cell body. The labeled cell bodies were analyzed for mRNA of the trk (high affinity) and low affinity (p75) neurotrophin receptors. The expression pattern of receptors differed for the nerves serving the 5 target fields. For example, trkA was expressed by 44 of 47 neurons to fungiform papillae and 36 of 39 cutaneous nerves to the ear. However, 25 of the 39 cutaneous nerves also expressed trkC, whereas none of the fungiform papilla nerves expressed this receptor. Moreover, only 6 of 42 and 4 of 44 nerves to incisive papilla and soft palate, respectively, expressed trkA. In summary each of the 5 target fields innervated by GG nerve branches had a unique pattern of neurotrophin receptor gene expression. Supported by NIH Grant # DC04837.

329 Slide : Development of Chemoreceptor Cells

RETINOIC ACID AND NEUROGENESIS IN POSTNATAL RAT OLFACTORY EPITHELIUM

Asson-Batres M., Smith W.B. *Biological Sciences, Tennessee State University, Nashville, TN*

Retinoic acid (RA) is essential for normal development of mammals. It is synthesized from vitamin A (VA), *all-trans-retinol*, through two enzyme-catalyzed steps. Available evidence indicates there are at least three retinaldehyde dehydrogenases, RALDH I, II, III, that catalyze the terminal step in this pathway. We have reported previously that RA is present in postnatal rat olfactory tissue, and that RALDH I is localized in sustentacular cells in the olfactory epithelium (OE) and in ensheathing cells that envelop olfactory axons in the underlying *lamina propria.* Using immunohistochemical detection, we show here that RALDH II is also expressed in cells adjacent to nerve fascicles. RALDH II colocalizes with the retinoid binding protein, CRBP I, but not RALDH I. We show further, that RALDH I colocalizes with the RA binding protein, CRABP II in sustentacular cells in the OE. These findings substantiate the prediction that RA is synthesized locally in the postnatal olfactory organ and suggest it may be involved in the regeneration of olfactory neurons. Using a dietary VA deficiency (VAD) model, we reported previously that RA is depleted in olfactory mucosa from postnatal VAD rats. Decreased expression of mature olfactory neuron markers was observed, along with an increase in the proliferation of cells in the basal region of VAD OE, suggesting that regeneration of functional neurons may be impeded in RA-deprived OE. We report here that a further consequence of RA deficiency is an increase in the number of GAP-43$^+$ immature neurons in VAD OE and GAP-43$^+$ axons in the underlying *lamina propria.* These findings suggest that RA may be involved in processes that promote terminal differentiation or maturation of olfactory neurons.

330 Slide : Development of Chemoreceptor Cells

AN INDUCIBLE TRANSCRIPT EXPRESSED BY REACTIVE EPITHELIAL CELLS THAT PROLIFERATE LOBSTER OLFACTORY SENSORY NEURONS

McClintock T.S.[1], Nickell M.D.[1], Stoss T.[1], Derby C.D.[2] *[1]Physiology, University of Kentucky, Lexington, KY; [2]Biology, Georgia State University, Atlanta, GA*

Olfactory sensory neurons (OSNs) are continuously renewed. In lobsters, the source of new OSNs is the ventral epithelium of a specialized region of the first antennule, the proliferation zone (PZ) (Steullet et al., 2000, J. Neurosci. 20:3282). The physical separation of this proliferative region from the mature zone allowed us to perform expression profiling by differential amplification. We obtained 12 fragments of transcripts that were many-fold enriched in the PZ compared to the mature zone. These transcripts include four novel sequences, a growth factor, three exoskeletal proteins, a serine protease, and two serine protease inhibitors. These transcripts were unaffected by stimuli that increase proliferation of OSNs. Some of them were expressed only in epithelial cells, which are the most abundant cells in the PZ. Others were expressed in epithelial cells and cells of the OSN lineage, consistent with the proposed lineage relationship between these cells. The final transcript, PET-15, was expressed only by epithelial cells, primarily in the PZ but also in damaged areas of the mature zone. Damage to the olfactory organ, which increases proliferation in the PZ, caused the abundance of PET-15 to increase many-fold in both the damaged ipsilateral PZ and the PZ of the undamaged contralateral control. This result is evidence that a diffusible signal controls the rate of OSN proliferation. Supported by NIH grants DC02366 and DC00312.

331 Slide : Functional Organization of Olfactory Systems

HUMAN SPECIFIC LOSS OF OLFACTORY RECEPTOR GENES

Yoav G.[1,2], Orna M.[2], Svante P[1], Doron L.[2] [1]*Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany;* [2]*Dept. of Molecular Genetics, Weizmann Institute, Rehovot, Israel*

Olfactory receptor (OR) genes constitute the basis for the sense of smell and are encoded by the largest mammalian gene superfamily of >1,000 genes. In humans, >60% of these are pseudogenes. In contrast, the mouse OR repertoire, although of roughly equal size, contains only ≈ 20% pseudogenes. We asked whether the high fraction of nonfunctional OR genes is specific to humans or is a common feature of all primates. To this end, we have compared the sequences of 50 human OR coding regions, regardless of their functional annotations, to those of their putative orthologs in chimpanzees, gorillas, orangutans, and rhesus macaques. We found that humans have accumulated mutations that disrupt OR coding regions roughly 4-fold faster than any other species sampled. As a consequence, the fraction of OR pseudogenes in humans is almost twice as high as in the non-human primates, suggesting a human-specific process of OR gene disruption, likely due to a reduced chemosensory dependence relative to apes.

332 Slide : Development of Chemoreceptor Cells

IDENTIFICATION OF MULTIPOTENT OLFACTORY PROGENITOR CELLS IN VITRO BY VIDEOMICROSCOPY AND IMMUNOCYTOCHEMISTRY

Cunningham A.M.[1], Marlicz W.[1] [1]*Developmental Neurosciences Program, Faculty of Medicine, University of New South Wales, Sydney, Australia*

Although the mammalian olfactory neuroepithelium (ON) is recognized as avidly supporting neurogenesis and capable of reconstituting cells of both neuronal and non-neuronal lineages, our knowledge of the olfactory neuronal progenitor cell remains limited. We aimed to isolate and characterize the neuronal progenitor of the rat ON *in vitro*. Progenitor cells were isolated from neonatal Wistar pups and enriched by selective filtration away from olfactory neurons (Cunningham et al. 1999). Using time-lapse videomicroscopy we identified motile cells that generated multicellular 'neurospheres' in primary culture. Immunocytochemical analysis of the spheres was performed with markers including nestin, cytokeratin, SUS-4, and GBC-1, and neuronal and glial markers. Olfactory spheres generated neurons and sustentacular cells *in vitro*. The neuronal progeny were of the olfactory lineage and showed a gradient of differentiation as they migrated away from the spheres, defined by expression of neuron-specific tubulin, Olf-1, olfactory G-protein (G_{olf}) and type III adenylate cyclase. Cells in the spheres were passaged and generated secondary spheres, although with less frequency than central forebrain neurospheres. Finding a progenitor cell that generates sustentacular and neuronal progeny is consistent with Huard et al. 1998, who proposed that the ON harbored multipotent progenitor cells. This *in vitro* system will allow us to further understand this unique neurogenic pathway. Supported by the Garnett Passe and Rodney Williams Memorial Foundation and the NH&MRC of Australia

333 Poster : Oscillations & Synchronization in Olfaction

GENERATION MECHANISM OF EOG OSCILLATIONS IN THE RAINBOW TROUT

Suzuki N.[1], Takahata M.[1], Shoji T.[2] [1]*Ani Behav & Intelli, Div Biol Sci, Grad Sch Sci, Hokkaido University, Sapporo, Hokkaido, Japan;* [2]*Sch Mar Sci & Technol, Tokai University, Shimizu, Shizuoka, Japan*

Oscillations superimposed on electro-olfactograms (EOGs) have been often observed in a variety of animals from fishes to mammals. Because of their variability of occurrence in EOG recordings in different animals with different stimulation methods, the generation mechanism of EOG oscillations has remained unclear. We have recently shown that current oscillations occur in whole-cell responses of isolated olfactory receptor neurons (ORNs) in the rainbow trout when ORNs are stimulated by odorants at high intensities (Suzuki et al, 2002). In the present study, we examined the characteristics of EOG oscillations induced by odorant stimulation with a quadruple mixture of amino acids (L-Glu, L-Arg, L-Ala and L-Nva). EOG oscillations processed with a digital high-pass filter at 2 Hz were analyzed by a continuous wavelet transform using the eighth-order Gabor function. The oscillations appeared mostly on the peak or decay phase of negative EOG responses and were recorded from ~50 % individuals. The peak amplitude and frequency of oscillations were 1.74 mV (n=126; range 0.12-8.29 mV) and 8.95 Hz (range 3.51-19.14 Hz) when stimulated with 10 mM mixture. The peak amplitude of oscillations was correlated with the amplitude of EOG responses. The occurrence of oscillations was dependent both on the stimulus concentration and flow rate. The peak frequency of oscillations increased significantly with increased stimulus flow rate. We are now developing a computer simulation program based on the assumption that EOG oscillations are local field potentials composed of the time-lagged summation of oscillatory receptor potentials of individual ORNs.

334 Poster : Oscillations & Synchronization in Olfaction

VOMEROPHERINS PRODUCE LOW-FREQUENCY CALCIUM OSCILLATIONS IN HUMAN VOMERONASAL NEURONS

Winegar B.[1], Monti-Bloch L.[1] [1]*Pherin Pharmaceuticals, Mountain View, CA*

The mammalian vomeronasal system detects pheromones released from conspecific individuals, with resultant neuroendocrine and behavioral responses. OBJECTIVES: In an effort to understand sensory coding in the human vomeronasal system, human vomeronasal neurons (VNs) were collected from volunteers and studied in vitro. Intracellular Ca was measured during perfusion with a series of pregnanes, norpregnanes and androstanes. METHODS: Human VNO neurons (VNs) were collected from human volunteers following an approved protocol. Cells were used within 24 - 48 hours of collection and most often within several hour of plating. VN cultures were incubated for 40 minutes at 36?C in Ringer's solution with 3 μM fura-2-AM (Molecular Probes). The cells were washed in fresh Ringer's, transferred to a perfusion chamber and images were captured at a rate of 1 Hz. Drugs were applied as 10 μM solutions unless otherwise noted. The experiments were divided into 100 s epochs to study the amplitudes and spectral distribution of intracellular Ca fluctuations, which allowed resolution of Ca signals from 0.01 to 0.2 Hz. RESULTS: Vomeropherins, like natural pheromones, are generally believed to be excitatory because they increase intracellular calcium levels. The panel of vomeropherins that we tested typically increased intracellular calcium ~100 – 200 nM. A number of vomeropherins also produced reversible increases in low-frequency calcium fluctuations. DHEA was without effect. Control experiments with 0.1% DMSO also had no effect on intracellular Ca. Calcium oscillations may be related to the molecular mechanisms of sensory transduction.

335 Poster : Oscillations & Synchronization in Olfaction

REDUCED EXTERNAL CALCIUM OR POTASSIUM CHANNEL BLOCKADE EVOKES BURSTING IN CRAYFISH PARASOL CELLS

Mellon D.[1] [1]*Biology, University of Virginia, Charlottesville, Virginia*

Crustacean parasol cells are multimodal local sensory interneurons within the hemiellipsoid body (HEB) and receive input from accessory lobe (AL) projection neurons, as well as from the ipsilateral compound eye. In perfused, isolated crayfish head preparations, parasol cells exhibit periodic (ca. 0.5 Hz), synaptically driven depolarizations from unidentified neurons in the terminal medulla.The functional significance of this periodic background activity is unknown. Strong sensory input via the compound eyes or projection neurons can generate trains of multiple bursts in parasol cells. I now report that reducing external calcium by substitution with magnesium, or addition of manganese or cobalt chloride to the calcium- containing perfusate, evokes spontaneous bursting in crayfish parasol cells. Addition of 10 mM TEA to normal saline perfusate also leads to spontaneous bursting, as does addition of 10 micromolar 5-HT to the saline. TTX (1 micromolar) suppresses background activity and overshooting spikes, but even after 25' of TTX treatment injected current generates spike-like activity in these neurons. I propose that in normal conditions continuous background activity may suppress spontaneous bursting, perhaps by maintaining high membrane potentials through calcium-gated, TEA-sensitive potassium currents. In other invertebrate preparations 5-HT is known to close both S-types and delayed rectifier potassium channels. Bursts may represent non-TTX-sensitive voltage-gated sodium channels. Currently, experiments are planned to determine whether a voltage-sensitive sodium pump is a possible mechanism for terminating bursts under conditions of divalent cation manipulation. Supported by NSF grant IBN9727753.

336 Poster : Oscillations & Synchronization in Olfaction

ODOR-INDUCED SYNCHRONY OCCURS AMONG CATFISH OLFACTORY BULB NEURONS HAVING SIMILAR ODORANT SELECTIVITIES IRRESPECTIVE OF THEIR BULBAR LOCATION WITHIN THE AMINO ACID CHEMOTOPIC ZONE

Nikonov A.[1], Caprio J.T.[1] [1]*Biological Sciences, Louisiana State University, Baton Rouge, Louisiana*

To determine whether synchronous odorant-induced responses occurred for neurons having the same odorant selectivities, simultaneous recordings of single olfactory bulb (OB) units and the associated local field potentials (LFPs) were obtained from paired sites within the amino acid chemotopic region. The two most numerous types of amino acid units [methionine (Met) and arginine (Arg)] within the OB were selected for this study. Excitatory responses to 10^{-6}M -10^{-4}M Arg and Met were obtained from OB neurons separated by $\leq$ 250 μm and > 500 μm. Responses to Met and Arg were often antagonistic or non-stimulatory to Arg and Met units, respectively. Odor-evoked action potentials from paired Met and paired Arg units were phase-locked to their LFPs with a significantly greater time lag for the Arg [24.1 ± 0.7 (S.E.)] msecs) than for the Met [18.5 ± 0.6 msec] units. Irrespective of distance, OB LFPs recorded at the two electrode sites were phase-locked with time lags whose 95% confidence intervals (CI) were $\leq$ 3.8msecs and overlapped zero (perfect synchrony) for paired Met or paired Arg units separated by > 500 μm. Since a defined relationship exists between the LFP and the production of action potentials by Met and Arg units, respectively, the phase-locking of the LFPs indicate epochs of odor-induced synchrony of action potentials of Met and of Arg units irrespective of distance of separation within the amino acid chemotopic region of the catfish OB. Supported by NIH DC03792.

337 Poster : Oscillations & Synchronization in Olfaction

PERSISTENT FAST OSCILLATORY ACTIVITY IN THE RAT OLFACTORY BULB IS MEDIATED BY CHEMICAL AND ELECTRICAL SYNAPSES IN VITRO.

Friedman D.[1], Strowbridge B.W.[1] [1]*Case Western Reserve University, Cleveland, OH*

The perception and discrimination of odorants is associated with fast (20 – 70 Hz) EEG oscillations recorded in the mammalian olfactory bulb or its insect analogue *in vivo*. In this study, we developed an in vitro model of odor-evoked oscillations to define the cellular mechanisms of this activity. We show that fast field potential oscillations can be evoked by brief tetanic electrical stimulation in the rat olfactory bulb *in vitro*, partially mimicking the natural response of this brain region to sensory input. Stimulation induces periodic inhibitory synaptic potentials in mitral cells and prolonged spiking in GABAergic granule cells recorded using whole-cell and extracellular recording techniques. Repeated stimulation leads to the persistent enhancement in field potential oscillations, granule cell activity and mitral cell inhibition. Prominent oscillations in field recordings indicate that stimulation induces high frequency activity throughout networks of olfactory bulb neurons. Network synchronization results from chemical and electrical synaptic interactions since both glutamate receptor antagonists (APV and NBQX, 18.8 ± 4.7 and 9.0 ± 4.9% of control, respectively; N= 7 and 4 slices; $p<0.01$) and gap junction inhibitors block oscillatory intracellular (e.g. halothane, 55.7 ± 17% of control; N= 7 mitral cells; $p<0.05$) and field responses (49.4 ± 9.7% of control; N= 7 slices; $p<0.05$). Our results demonstrate that the olfactory bulb can generate fast oscillations autonomously through the persistent activation of networks of inhibitory interneurons.

Supported by NIH (DC04285 & NS33590).

338 Poster : Oscillations & Synchronization in Olfaction

ODOR-INDUCED OSCILLATIONS IN RAT OLFACTORY BULB

Scott J.W.[1], Sherrill L.[1] [1]*Cell Biology, Emory University, Atlanta, GA*

Oscillatory odor-driven field potentials have long been considered important markers of the timing of the activity of mitral/tufted cell and interneuron discharge in the olfactory bulb. We have begun investigating these relationships in rats anesthetized with urethane (1.5 gm/kg). Recordings have been made with glass micropipettes or with silicon microelectrode arrays (4x4 and 1x16). Oscillatory field potentials have been evoked by a number of odorants, but most effectively by esters. These field potentials were often highly synchronous even when recorded by two or more electrodes in very distant parts of the olfactory bulb, suggesting that activity is often volume conducted through much of the bulb. For this reason, we have applied current source density analysis to find sites where oscillatory field potential responses are generated. We have found that very large field potential oscillations can be observed that do not invert at the mitral cell layer and are not associated with detectable current sinks or sources. In other cases neighboring electrode arrays show substantial differences in current density measurements when there is little difference in the size of the field potential. Preliminary results indicate that very few single cell spikes are in synchrony with either the field potential or current density records although many cells appear to have rhythmic activity. We conclude that great care must be taken in suggesting that field potential oscillations in the bulb truly represent local neural activity at the site of the electrode. Supported by NIH grant DC00113

339 Poster : Olfactometry & Gustometry

A TEMPORAL AUTOMATED SYSTEM FOR TASTE EXPERIMENTS

Ashkenazi A.[1], Buckley J.[2], D'Alessandro T.[2], Dirubba A.[2], Fritz M.[2], Goodman R.[2], Marks L.E.[1] *[1]J. B. Pierce Laboratory and Yale University, New Haven, CT; [2]J. B. Pierce Laboratory, New Haven, CT*

Precise temporal control of stimulation is important to psychophysical research in general and crucial to experimental studies of attention. In studies of attention particularly, traditional methods used in taste experiments, such as sip and spit, do not provide either temporal precision or the ability to present brief stimuli in close succession. To extend our program of research into the mechanisms underlying gustatory attention, we have designed a computer-controlled, automated, open flow system. This system provides precise temporal control over relatively a large number (up to 16) of possible stimuli. Using pressurized air to control delivery of the solutions, through an interface containing 16 nozzles, the system makes it possible to present to the tongue stimuli of short as well as longer duration, and to provide short or long intervals between stimuli and water rinses. Calibrations show relatively precise and reliable control of temporal properties of the stimuli, and initial measurements of taste sensitivity permit comparison of psychometric functions obtained with this system to functions obtained by sip and spit. This research was supported by NIH grant 5R01 DC00271-16 to LEM.

340 Poster : Olfactometry & Gustometry

HOW TO MAKE AND MEASURE SMELLS - PRACTICAL LESSONS IN OLFACTOMETRY

Schmidt R.[1], Cain W.S.[1] *[1]Chemosensory Perception Laboratory, Surgery (Otolaryngology), University of California, San Diego, La Jolla, California*

A workshop on olfactometry will include presentation of the VDD-8, an 8-station vapor delivery device with many advantages for psychophysical testing. Goals for its creation included: 1) Simultaneous availability of up to 8 levels of concentration. 2) A wide range of concentrations, with flexibility in step-size between them. 3) Up to 3 choices per station for testing via 3-alternative forced-choice. 4) Capacity to test 8 subjects simultaneously. 5) Generation of vapor from neat material in most instances, though with the flexibility to start with any form. 6) Intuitive interface between operator and device. 7) Environmentally realistic interface between device and subject. 8) Automatic commands to prompt subjects through testing. 9) Practicability of calibration. 10) Uncomplicated maintenance and relative ease of repair. The VDD-8 has shown reliability and adaptability in hundreds of tests with a variety of test materials, ranging from the reactive to the nonreactive, and from the highly volatile to the almost involatile. This presentation will show the device in detail, explicate the attainment of the goals, and illustrate the routines necessary to operate it. The lessons will be applicable to a wide range of devices. They will include the design of spreadsheets: a) to allow an operator to anticipate delivered concentrations and to avoid condensation from test material or solvent, b) to set up circumstances for semi-automatic or automatic testing, and c) to perform absolute calibrations of delivered concentrations. Supported by Union Carbide Corp., a division of Dow Chemical Co.

341 Poster : Olfactometry & Gustometry

DEVELOPMENT OF A PRECISION OLFACTOMETER

Hastings L.[1], Doty R.L.[2] *[1]Sensonics, Inc., Haddon Heights, NJ; [2]Smell and Taste Center, University of Pennsylvania, Philadelphia, PA*

Many scientific and industrial applications require the presentation of precise, repeatable, and quantifiable olfactory stimuli. Currently, no standardized olfactometer for generating such stimuli is produced commercially within the United States. Consequently, we have developed a precision olfactometer suitable for research in the scientific, medical, and industrial communities. This instrument uses permeation tubes as the initial source for accurate and stable generation of olfactory stimuli. A digital dry bath incubator is used to maintain the odor stimuli at a constant temperature and mass flow controllers are used to precisely regulate the flow of air. Up to four different odor stimuli can be generated; each individual stimulus can be varied in concentration approximately two log steps. To further extend the range of available concentrations, permeation tubes differing in output can be placed in the four channels. The odor stimuli are conveyed to the subject via Teflon lines enclosed in a flexible PVC tube and are maintained at a constant temperature by a flexible heating tape located within the tube. The odor lines terminate into a Teflon manifold located at the end of the PVC tube. Since the odor stimuli are added to the main carrier airflow just before it reaches the subject, onset and offset of the stimulus presentation occurs very rapidly. This olfactometer can generate accurate and reproducible odor stimuli at concentrations of threshold and suprathreshold levels, is easy to operate and maintain, and is cost effective. Supported by DC 04024 from NIDCD-NIH.

342 Poster : Olfactometry & Gustometry

NEW TECHNIQUE IN REAL-TIME MONITORING OF CHEMOSENSORY (GAS) STIMULUS FOR EVENT-RELATED POTENTIALS

Kobayakawa T.[1], Toda H.[1], Yamada H.[1], Saito S.[1] *[1]Neuroscience research institute, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan*

In 1993, Evans et al. proposed suggestions for collection and reporting of chemosensory event-related potentials (CSERPs). They described that chemosensory stimuli should be embedded in a constant flow and the rise-time of stimuli should be less than 50 ms to 70 % of maximum concentration. And it also suggested two means for observation of replacement from air to odorant. One method was usage of photo-dense aerosol instead of direct odorant and the other was that of temperature change. These methods, however, did not directly measure the replacement from air to odorant. Additionally, ordinal thermistor and thermocouple do not trace milli second change, because of the time lag of first order in thermal conduction. The other observation method, using aerosol, will pollute one of odorant lines of olfactometer usually, which decreases possible variation of odor stimuli. In order to solve these problems, we have originally developed an ultrasonic gas sensor. This sensor is possible to directly monitor the real-time change of odorant concentration, in every 0.5 ms (sampling rate 2kHz), which is sufficiently rapid to observe stimulus rise time. Using this technique, we have measured the CSERPs without any artifact, with simultaneous observation of replacement from air to odorant. The averaged rise-time (n = 40) was 48 ms to 70% of maximum concentration. And rise-time became longer in latter trials. We also confirmed this sensor did not affect magnetoencephalography (MEG) signal.

343 Poster : Olfactometry & Gustometry

IMPROVEMENTS TO AN OLFACTOMETER FOR PSYCHOPHYSIOLOGICAL AND FMRI STUDIES OF OLFACTORY PROCESSES

Lorig T.[1], Gould M.[2], Dalton P.[2] [1]*Psychology, Washington and Lee University, Lexington, VA;* [2]*Monell Chemical Senses Center, Philadelphia, PA*

Previously, Lorig, Elmes, Zald and Pardo described a simple odor delivery device for use in fMRI and psychophysiological experiments. The device, under computer control, passed an air stream over filter paper impregnated with odor material. This was accomplished without perceptible changes in air flow. The improvements we describe are related to the construction, size, and day to day use of the device. The new olfactometer design is easy to construct, highly reliable, and can be easily filled and refilled with odorants. In addition, the new design makes it possible to simultaneously monitor nasal air flow during odor administration. Supported by grants from KAO Corporation and NIH (R01 DC 03708 to PD)

344 Poster : Olfactometry & Gustometry

SINGLE-PASS ENVIRONMENTAL CHAMBER FOR QUANTIFYING HUMAN RESPONSES TO AIRBORNE CHEMICALS

Suarez J.C.[1], Warmath D.S.[1], Koetz K.P.[1], Hood A.F.[1], Thompson M.L.[1], Kendal-Reed M.S.[1], Walker D.B.[1], Walker J.C.[1] [1]*Sensory Research, Florida State University, Tallahassee, Florida*

Despite increasing interest in the short-term effects of airborne environmental contaminants, experimental findings are generated at a very slow pace. This is due in part to the expense and complexity of most environmental chambers, which are needed for quantifying effects of whole-body exposures. We lessened this obstacle by designing, constructing and testing a single-pass 10-m^3 stainless steel chamber. Compressed air is purified before being sent to an air dilution olfactometer which supplies 1000 L (1 m^3) per minute and maintains 50% RH and 22.5° C. Precise control of all stimulus parameters is greatly simplified since air is not recirculated through an external ventilation system. Odorant concentrations are achieved by varying the proportion of total airflow passing through one or more saturators, and are verified in real-time by an IR spectrometer. An adjoining 5-m^3 anteroom is used for introducing known intensities of more chemically complex vapor and/or particulate stimuli into the chamber. Prior to the point that air is exhausted from the chamber, all components are made of stainless steel, Teflon or glass. A LabView program contains feedback loops that achieve and document desired chamber conditions. Additional instrumentation and computer systems provide for the automated collection of perceptual, respiratory, eye blink, heart rate, blood pressure, psychological state and cognitive data. This system is now being employed to investigate the relative importance of different chemosensory inputs in mediating these kinds of responses to a frequently encountered complex mixture: second hand smoke. Supported by research funds from the Philip Morris External Research Program.

345 Poster : Vomeronasal Chemoreception

HUMAN V1-SUBTYPE VOMERONASAL RECEPTORS

Sangameswaran L.[1], Edwards D.[1], Tran L.[1], Raghu G.[1] [1]*Pherin Pharmaceuticals, Mountain View, CA*

Pheromones are chemosensory substances that are believed to elicit physiological and/or behavioral effects through receptor-mediated mechanisms in sensory neurons localized in the vomeronasal organ (VNO). Our goal is to isolate and characterize the vomeronasal receptors (VR) expressed in the human VNO. In the rodents, the sensory receptors in the VNO have been characterized as members of the G-protein coupled receptor (GPCR) gene family that have a characteristic 7 transmembrane domain. These vomeronasal receptors apparently fall into 2 families: V1 and V2. One hundred and thirty seven members of the V1 subtype have been identified and by phylogenetic analysis these genes fall into 12 subfamilies: V1a-V1l. To identify human homologs of V1-subtype genes, we performed similarity-based iterative searches of the Human Genome Database in the public domain. By using different mouse and rat V1/3-subtype VR sequences as baits, a large number of sequences were analyzed for potential contiguous open reading frames that preserved all the characteristic features of this gene family. Putative receptor sequences were identified as intronless coding regions. These were cloned by PCR amplification methods out of human genomic DNA. The expression profile of these putative VRs was assessed in a variety of human tissues including the VNO, olfactory region and brain regions by RT-PCR.

346 Poster : Vomeronasal Chemoreception

SPECIES-SPECIFICITY IN PHEROMONE RECEPTOR LOCI?

Lane R.P.[1], Cutforth T.[2], Axel R.[2], Hood L.[3], Trask B.J.[4] [1]*Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT;* [2]*Biochemistry and Molecular Biophysics, Columbia University, New York, NY;* [3]*The Institute for Systems Biology, Seattle, WA;* [4]*Fred Hutchinson Cancer Research Center, Seattle, WA*

We have analyzed the organization and sequence of 73 V1R genes encoding putative pheromone receptors at three mouse genomic loci. These 73 V1Rs arose from seven ancestral genes by large local duplications. These duplications are correlated with surrounding Line1 retroelement repeat activity and appear to have occurred over a narrow ~10 MY time window approximately 25 MYA. This period overlaps major murine speciation events and raises the possibility that repeat-mediated V1R expansions may have contributed to species divergence.

347 Poster : Vomeronasal Chemoreception

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF VOLTAGE-ACTIVATED PROPERTIES IN VOMERONASAL NEURONS ISOLATED FROM ADULT MALE AND FEMALE MICE

Dean D.[1], Mazzatenta A.[1], Menini A.[1] *[1]SISSA (Scuola Internazionale Superiore di Studi Avanzati), Trieste, Italy*

The vomeronasal organ (VNO) is used by many animals to register chemical cues such as pheromones produced by other individuals of the same species, as indications of the physiological status of these individuals. Via the VNO and its target organs in the brain (together called the Accessory Olfactory System (AOS)), pheromones have a strong influence on the endocrine system and therefore on the regulation of reproductive function and behavior. Of interest in the present study, is that normal VNO function has been shown to confirm sex-specific behaviors on individuals of a given species. Where then, is the earliest point in the AOS where a discrepancy between males and females may be found? This question and a lack of electrophysiological data from the mouse VNO drove us to explore the possibility of a sexual dichotomy at the level of voltage-activated properties of the individual VNO neuron. We used the patch-clamp technique to study neurons isolated from adult males and females of two strains of mice (BALB-c and CBA). Cells were voltage-clamped around their resting membrane potentials while whole-cell currents were measured in response to voltage steps of various durations. Inward and outward current properties were examined in detail while averaged data from both sexes were subjected to statistical analysis. Preliminary results suggest that, independent of the engagement of the transduction cascade of these cells, there is no clear or significant sexual dichotomy among the measured voltage-activated properties of mice VNO sensory neurons.

348 Poster : Vomeronasal Chemoreception

THE ROLE OF GQ/11 IN SIGNAL TRANSDUCTION IN THE VOMERONASAL ORGAN

Miller S.[1], Napier A.[1], Wekesa K.[1] *[1]Biological Sciences, Alabama State University, Montgomery, AL*

The social behaviors of most mammals are affected by pheromones. Pheromone detection is mediated by the vomeronasal system, which includes the vomeronasal organ (VNO) located in the nasal cavity and the accessory olfactory bulb located in the brain. More specifically, pheromones are detected via G-protein coupled receptors located within the VNO. To investigate the role of Gαq/11 in this signal transduction pathway, microvillar membrane preps from murine VNO were isolated from prepubertal females. These membranes, were incubated with adult male urine and resulted in an increase in production of inositol-(1,4,5)-trisphosphate (IP3). This stimulation is mimicked by GTPγS, blocked by GDPβS and is tissue specific. Immunohistochemical studies with monospecific antibodies reveal the presence of three G-proteins: Gαo, Gαi2 and Gαq/11-related protein in vomeronasal neurons, concentrated at their microvilli. Use of bacterial toxins that lead to ADP-ribosylation of the G-protein alpha subunits of Go and Gi2 do not block the increase in IP3. However, the use of U-73122, a PLC inhibitor blocks the production of IP3. Our results indicate that pheromones in male urine act on vomeronasal neurons in the female VNO via a receptor-mediated Gαq/11-protein dependent increases in IP3 levels. This work was supported by a grant from the National Institutes of Health (GM08219) to KSW.

349 Poster : Vomeronasal Chemoreception

IMMUNOHISTOCHEMISTRY OF THE CANINE VOMERONASAL ORGAN

Dennis J.C.[1], Algier J.G.[2], Morrison E.[3] *[1]Anatomy, Physiology, Pharmacology, Auburn University, AL; [2]College of Veterinary Medicine, Auburn University, [3]Anatomy, Physiology, Pharmacology, Auburn University*

The canine's olfactory acuity is legend but its olfactory system is not well described. We used immunohistochemistry on paraffin embedded sections of male and female adult dogs to characterize the expression of proteins known to be expressed in the vomeronasal organ of several other species. Basal cells were more apparent in each section compared to rodent vomeronasal organ and expressed immunoreactivity to cytokeratin and epidermal growth factor receptor. The thin layer of sensory neurons and axon fascicles in the lamina propria expressed neuron cell adhesion molecule. Many sensory neurons coexpressed neuron-specific beta tubulin and protein gene product 9.5. Some neurons expressed growth associated protein 43 and a number of those also expressed neuron-specific beta tubulin. Axon fascicles were double labeled for those two proteins. The G-protein alpha subunits Gi and Go, involved in the signal transduction pathway, showed immunoreactivity in the sensory cell layer. Our results demonstrate that the canine vomeronasal organ contains a population of cells that express several neuronal markers expressed by VNO sensory neurons in other species. Furthermore, growth associated protein 43 immunoreactivity suggests that the sensory epithelium is neurogenic in adult dogs.

350 Poster : Vomeronasal Chemoreception

A 20 KD PROTEIN COMPONENT OF MALE *PLETHODON SHERMANI* PHEROMONE ACTIVATES FEMALE *P. SHERMANI* VOMERONASAL NEURONS

Wirsig-Wiechmann C.R.[1], Houck L.D.[2], Feldhoff P.W.[3], Shaikh A.[1], Feldhoff R.C.[3] *[1]Cell Biology, University of Oklahoma, Oklahoma City, OK; [2]Zoology, Oregon State University, Corvallis, OR; [3]Biochemistry and Molecular Biology, University of Louisville, Louisville, KY*

We have previously shown, using agmatine uptake as an indicator of neural activation, that male Plethodon shermani salamanders produce a pheromone (extracted from mental glands) that activates female vomeronasal neurons (VNNs). This pheromone extract, when placed on the female's snout, increases the female's receptivity to the male during courtship. The major protein component of this pheromone extract, a 20 Kd protein termed plethodon receptivity factor or PRF, also increases female receptivity. We now report that PRF also activates a population of female VNNs. Females screened for positive courtship behavior received 20 ul of 3 mM agmatine in saline with (n=5) or without (n=5) PRF over a 40 minute period. Frozen sections of whole salamander heads were immunocytochemically labeled to visualize neurons that contained agmatine. Labeled cells were quantified across the entire vomeronasal organ. Vomeronasal organs that received PRF contained an average of 327 labeled VNNs, whereas those that received saline contained an average of 76 labeled cells. This difference was statistically significant (Unpaired Student t, $t=5.7$, $df=8$, $P<0.0002$). This study has demonstrated that the major protein component of male P. shermani pheromone that elicits an increase in female receptivity also causes neural activation within the female's VNNs. Supported by the National Science Foundation IBN-0110666.

351 Poster : Vomeronasal Chemoreception

A PROTEOMIC ANALYSIS OF MUCOSAL PROTEINS IN THE VNO DUCT OF THE ASIAN ELEPHANT: CONTINUING THE HUNT FOR PHEROMONE BINDING PROTEINS.

Greenwood D.R.[1], Rasmussen L.E.[2] [1]*Gene Technologies, HortResearch, Auckland, New Zealand;* [2]*Oregon Graduate Institute of Science & Technology, Beaverton, Oregon*

Pheromone molecules trudge a tortuous path on their journey from source-to-sink. In previous studies we have demonstrated that specific proteins facilitate the sequential transport of two pheromonal compounds from their respective sites of synthesis to reception in the vomeronasal organ (VNO) of the Asian elephant *(Elephas maximus)*. Both *(Z)*-7-dodecen-1-yl acetate (the female-to-male sex pheromone) and the bicyclic ketal, frontalin (the chemoindicator for the maturing musth state) are liganded to specific proteins in a pH-dependent fashion. Elephants use their vomeronasal olfactory system, via the flehman behavioral response, to monitor the flux of these compounds in body secretions enabling them to assess the state of estrus or musth respectively. Pheromone molecules sequestered in trunk mucus are presented to the opening of the VNO ducts during flehmen. Recent experiments have suggested that a protein present in the VNO duct is involved in binding pheromonal ligands for the final journey from ductal opening to the receptor milieu, a distance of over 20 cm along the duct. We have now used *de novo* sequencing analysis of data generated from electrospray MS-MS of tryptic digests of mucosal proteins isolated from 2-D gels of VNO mucus. This has enabled us to resolve and identify individual proteins from the molecular weight region known to bind radiolabelled pheromone molecules. Funded by NIH Grant RO1-DC03320

352 Poster : Vomeronasal Chemoreception

HUMAN VOMERONASAL ORGAN CHANGES DURING THE MENSTRUAL CYCLE

Monti-Bloch L.[1], Linder C.[1], Allen D.[1] [1]*Physiology/Pharmacology, Pherin Pharmaceuticals, Mountain View, CA*

The human vomeronasal organ detects pheromones released from conspecifics, with resultant brain activation and neuroendocrine, autonomic and behavioral responses. Gonadotropins have been shown to influence mammalian VNO growth. OBJECTIVE: The aim of this study was to compare the human VNO pit size in healthy menopausal and premenopausal women during different phases of the menstrual cycle with serum levels of gonadotropic and gonadal hormones. METHODS: Upon (IRB) signed consent both nasal passages of 32 menopausal women (55-65 y.o) and 165 premenopausal women (20-45 y.o.) of different ethnicity were clinically examined with the aid of a rigid nasoscope connected to a video camera. VNO images were computer-captured, stored and measured offline. In premenopausal women the procedure was performed consecutively during the early follicular (days 2-5), periovulatory (days 10-14) and luteal (days 23 – 25) phase of the menstrual cycle. Menopausal women were studied twice every 14 days. Serum FSH, LH, estradiol and progesterone were measured to assess cyclicity and hormonal status of the subject. RESULTS: In premenopausal women the VNO pit changed size during the menstrual cycle. We found significantly increased VNO pits in the periovulatory phase of the cycle as compared to the other phases. Menopausal women showed VNO pits significantly larger than those found in the follicular and luteal phase of the premenopausal group (control). These changes coincided with increased serum levels of gonadotropins. The changes in human VNO size reported here are interpreted as a result of the tropic effect of gonadotropic hormones on VNO tissue.

353 Poster : Vomeronasal Chemoreception

RELAXATION OF SELECTIVE PRESSURE ON AN ESSENTIAL COMPONENT OF VOMERONASAL TRANSDUCTION DURING PRIMATE EVOLUTION

Liman E.R.[1], Innan H.[1] [1]*Biological Sciences, University of Southern California, Los Angeles, California*

The vomeronasal organ (VNO) has been thought to be vestigial in humans. The gene encoding a key signaling component of VNO sensory transduction, the ion channel TRPC2, is a pseudogene in humans. TRPC2 is expressed exclusively in the VNO, and thus the accumulation of deleterious mutations within the coding sequence of TRPC2 most likely reflects the relaxed selective pressure on a functional VNO. To gain insight into where in primate evolution a functioning VNO was lost, we determined the time at which mutations in the TRPC2 gene occurred within the primate lineage. Of the six deleterious mutations in the TRPC2 gene, the first arose after the split between Old World (OW) and New World (NW) monkeys, and before the radiation of OW monkeys from apes. By analyzing the rate of synonomous and nonsynonomous substitutions in extant species and in deduced ancestral sequences, we find that there has been high purifying selective pressure on the TRPC2 gene in rats, mice and a prosimian primate, the lemur (Ka/Ks ~0.08), consistent with the known functional importance of the VNO in these species. Selective pressure was also high in the common ancestor of OW and New World (NW) monkeys. We find no evidence for selective pressure on the gene in any extant species of OW monkey or ape, including humans. These data point to a time in primate evolution after the divergence of NW monkeys from OW monkeys and apes where selective pressure on components of VNO sensory transduction was relaxed. Supported by NIDCD R01DC04213

354 Poster : Vomeronasal Chemoreception

ADULT HUMAN VOMERONASAL EPITHELIUM EXPRESS MOLECULAR MARKERS OF NEURON-LIKE ACTIVITY

Diaz V.[1], Morales A.[1], Castell A.[2] [1]*Reproductive Biology, National Institute of Medical Sciences and Nutrition, Mexico, DF, Mexico;* [2]*School of Medicine, National Autonomous University of Mexico, Mexico, DF, Mexico*

Functionality of the human vomeronasal organ (VNO) has been demonstrated by electrophysiological and pharmacodynamic studies. There is still controversy on the nature of the signal transduction pathway from the putative receptor site to the brain. OBJECTIVE: The aim of this study was to collect immunohistochemical data on the neurogenic character of the vomeronasal epithelium in humans, using specific antibodies against Chromogranin A (CgA), Synaptophysin (Syn), Nerve Growth Factor (NGF) and signal-transducing receptor proteins for neuroptrophins (Trk prot). METHODS: VNO samples were obtained from surgical samples. Subjects were informed of the purpose of the study and agreed to donate their tissue. Antibodies against Trk proteins were polyclonal that map within the intracytoplasmic domain. Antibodies against CgA, Syn and NGF were monoclonals that map within the external or the cytoplasmic domains. RESULTS: The epithelium lining of the VNO displayed a typical pseudostratified architecture composed mainly of bipolar cells that expressed a strong CgA and Syn immunoreactivity. Some sections of the VNO epithelium were positive for both NGF and Trk A indicating a neuron-like activity of at least a subset of bipolar cells. CONCLUSSION: The epithelium lining of the VNO is in a continuous cell replacement, during the adult life span, similar to other sensory epithelia and cell proliferation is under the control of trophic factors. Specific staining with Syn and NGF in the vomeronasal epithelia, strongly suggests the presence of active mature vomeronasal neurons. FUNDING. National Institute of Medical Sciences and Nutrition Salvador Zubirán, and National Autonomous University of Mexico

355 Poster : Human Olfaction: Psychophysics

THE LONG TERM EFFECT OF NASAL DILATORS ON NASAL ANATOMY AND OLFACTORY ABILITY

Lyng G.D.[1], Hornung D.E.[1], Leopold D.A.[2], Irwin S.B.[3], Vent J.[2]
[1]Biology Department, St. Lawrence University, Canton, NY; [2]Otolaryngology-Head and Neck Surgery, University of Nebraska Medical Center, Omaha, NE; [3]Otolaryngology-Head and Neck Surgery, Oregon Health Sciences Center, Portland, OR

Previous work has demonstrated that the immediate effect of nasal dilators is to increase the volumes of the soft nasal area and the bony nasal aperture in front of the olfactory cleft, decrease olfactory threshold and increase odor intensity ratings. To assess the temporal aspects of these changes, MRI imaging and olfactory testing were completed in the undilated condition, immediately after the dilator was applied, after the dilator had been in place for 4 hours and then after the dilator had been removed. Immediately after the nasal dilator was applied the volumes of both the soft nasal area and the bony aperture increased by 21% and 8% respectively. After four hours the volume of the bony aperture had increased by an additional 18% whereas there was only a modest further increase in the size of the soft nasal area. After the dilator was removed the volume of the bony aperture remained high whereas the volume of the soft nasal area returned to the undilated condition. The immediate effect of the nasal dilator was to decrease the olfactory threshold (PEA) by 6 binary dilution steps and increase odor intensity ratings by 40%. Both these measures of olfactory function remained elevated after 4 hours, and although there was some return toward the undilated condition, both remained elevated after the dilator was removed. These results suggest that the nasal reflex that changes the size of the bony aperture may have a relatively long time course and that changes in the volume of the bony nasal aperture may be important in determining olfactory function.

356 Poster : Human Olfaction: Psychophysics

CHARACTERIZATION OF THE SNIFF MAGNITUDE TEST

Niergarth K.A.[1], Dulay M.F.[2], Gesteland R.C.[3], Frank R.A.[2] *[1]Emerging Concepts, Inc., Cincinnati, OH; [2]Dept. of Psychology, University of Cincinnati, Cincinnati, Ohio; [3]Dept. of Cell Biology, University of Cincinnati, Cincinnati, Ohio*

A measure of olfactory function based on the reduction of the size of a sniff in response to a malodor, the Sniff Magnitude Test (SMT), is described. A series of experiments were conducted to assess the choice of malodors for the test and to address methodological concerns associated with the procedures used with the SMT. Four hundred and sixty-one university students were tested in four experiments. In Experiment 1, assessment of the psychophysical functions for a series of concentrations of methylthiobutyrate (MTB), ethyl mercaptoproprionate (EMP), and pyridine at ½ log dilution steps generally indicated that the malodors effectively suppressed sniffing until a precipitous drop in suppression at 0.1% v/v for all odorants. In Experiment 2, assessment of hedonic ratings and sniff magnitude in response to MTB, pyridine, and EMP at matched intensities indicated that MTB suppressed sniffs more effectively and was rated more unpleasant than the other malodors, but it was not rated as more intense. In Experiment 3, using 2 different procedures with repeated exposure to MTB, neither adaptation or habituation were observed at a 10 second inter-trial interval over as many as 12 consecutive trials. In Experiment 4, SMT procedures were changed to incorporate multiple malodors at different concentrations. Results indicated that all malodors were effective, but MTB was the most effective at identifying anosmics (as defined by UPSIT performance). The information from these studies was used to develop a more efficient and effective SMT procedure that is brief (3 minutes) and uses multiple malodors. Supported by NIH DC04139-02 to RCG.

357 Poster : Human Olfaction: Psychophysics

THE SNIFF MAGNITUDE TEST: A SENSORY MEASURE MINIMALLY INFLUENCED BY COGNITIVE FACTORS

Dulay M.F.[1], Niergarth K.A.[2], Shear P.K.[1], Frank R.A.[1], Gesteland R.C.[3]
[1]Psychology, University of Cincinnatii, OH; [2]Emerging Concepts, Inc., Cincinnati, Ohio; [3]Cell Biology, University of Cincinnati, OH

Olfactory performance is known to be related to the degree of cognitive impairment in older adults. However, it is unclear to what extent current clinical olfactory methods allow researchers to differentiate true olfactory losses due to the effects of aging or disease from an inability to meet the task demands due to cognitive limitations. The primary aim of this study was to assess the contribution of cognitive ability to performance on three olfactory measures with varying cognitive complexity. Eighty-four older adults (age range 59 to 93 years, education range 0 to 18 years) completed the Smell Identification Test (SIT), the Phenyl Ethyl Alcohol threshold task (PEA), the Sniff Magnitude Test (SMT) and a battery of neuropsychological tests. Poor performance on the SIT was related to reduced performance on tests of verbal comprehension ($r=.33$, $p=.002$), working memory ($r=.31$, $p=.004$), naming ($r=.39$, $p=.001$), and the Dementia Rating Scale-2 (DRS-2; $r=.38$, $p<.001$). Poor performance on the PEA task was associated with reduced performance on measures of attention ($r=.26$, $p<.02$) and the DRS-2 ($r=.22$, $p<.05$). Correlations remained significant when age and education were partialled out of the analyses. In contrast, the SMT was not significantly related to any of the measures of cognition. Results suggest that measures of olfaction dependent on the ability to recognize odors or discriminate between odor intensities may overestimate olfactory loss when cognitive limitations are not taken into account. The SMT, however, which requires only that participants sniff odorants, is less dependent on cognitive processes. Supported by NIH grants AG20446-02 to MFD & DC04139-02 to RCG

358 Poster : Human Olfaction: Psychophysics

DETECTION LATENCY FOR GOOD AND BAD SMELLS

Jacob T.J.[1], Townsend J.[1], Wang L.[1] *[1]School of Biosciences, Cardiff University, Cardiff, United Kingdom*

Since bad smells relay more urgent information than good smells we hypothesized that the olfactory system may detect bad smells more rapidly than pleasant smells. We therefore designed an experiment to test this hypothesis. The subjects, aged 18-25yrs (7 female, 6 male), were given the task of detecting odour pulses of different strengths delivered by a nasal canula to one nostril at 10 sec intervals. Odour strength was altered by varying the pulse duration. Blocks of 15 pulses of amyl acetate (AA; pleasant odour) and isovaleric acid (IVA; malodour) were alternated with 2 minute rests between blocks. When an odour pulse was detected subjects were required to press a button. The frequency of correct responses and the latency to detection were recorded. The response latencies were plotted as a histogram for each odour strength. The data were fitted with a binomial function and the mean, variance and skewness were determined for each distribution. The mean latencies ranged from 0.95.±0.02msec to 1.16.±0.05msec for IVA and 0.90.±0.02msec to 1.42.±0.05msec for AA. There was a greater positive skew for the AA data than for isovaleric acid. Positive skew indicates that the distribution is not symmetrical and is dispersed towards the right tail. Thus, for AA subjects gave more longer latencies than IVA. When the mean of the response latencies was plotted against detection frequency, thereby correcting for odour intensity, the latency for IVA was shorter than AA at all but the highest detection levels, where the two curves converged. Thus the initial hypothesis is proved, a malodour is detected faster than a pleasant odour.

359 Poster : Human Olfaction: Psychophysics

ADAPTATION TO GOOD AND BAD SMELLS

Jacob T.J.[1], Wang L.[1], Fraser C.[1], O'Connor S.[1] *[1]School of Biosciences, Cardiff University, Cardiff, United Kingdom*

Odour discrimination is important since pleasant and unpleasant smells require different behavioural responses. Bad smells warn us of danger which may require some immediate decision to be made and action to be taken, for example avoidance or withdrawl. Pleasant smells, on the other hand, do not necessitate immediate actions or decisions. The detection and processing of malodours is thus more urgent and therefore one might predict that the dose-response relationships and adaptation/habituation kinetics would be different for malodours versus pleasant odours. We therefore designed an experiment to test this hypothesis. The subjects, 18-25 year old students (5 female, 5 male), were given the task of detecting odour pulses of different strengths and frequencies delivered by a nasal canula to one nostril at 10 s intervals. Odour strength was altered by varying the pulse duration. Blocks of 15 odour pulses with 2 min rests between blocks were given. The chosen malodours were valeric acid, skatol, butyric acid and the pleasant odours were amyl acetate, cis-3-hexenol, linalool. When an odour pulse was detected subjects were required to press a button. We then determined the interstimulus interval (ISI) at which detection was half-maximal – ISI(50). The ISI(50) varied over a much greater range for malodours (3.0-8.5 sec for valeric acid) than pleasant odours (3.1-3.8 sec for hexenol) according to stimulus strength. Thus the human olfactory system adapts more readily to, and is more sensitive to concentration changes of, malodours than pleasant odours.

360 Poster : Human Olfaction: Psychophysics

CONTRASTING ACTIVATION OF HUMAN BRAIN BY COMPLEX DESIGNED FRAGRANCES

Yang Q.X.[1], Wang J.[1], Eslinger P.J.[2], Smith M.B.[1], Ansari R.[3], Richardson A.[3], Behan J.[3] *[1]Radiology, Pennsylvania State University, Hershey, PA; [2]Neurology, Pennsylvania State University, Hershey, PA; [3]Technical Affairs, Quest International Fragrance Company, Mount Olive, NJ*

Fragrances have been widely used to modulate the emotional state of mind and body across all cultures. There has been no comparative study of human affective brain activities induced by fragrances designed to elicit specific emotional reactions. We used functional MRI to investigate brain activations by two commercially popular fragrances that cause distinctive emotional reactions that are invigorating (type ST1221, Quest International) or relaxing (type SC1139, Quest International). Seven healthy right-handed young subjects (25.0 ± 2.2 yo, 4m/3f) received fMRI on a 3T system. T2* weighted EPI images were acquired during execution of the fMRI paradigm, which consisted of 5 cycles of alternating rest (45 sec) and olfactory stimulation (11 sec). Each subject received two olfactory fMRI scans with the two fragrances separated by a 12-minute interval. The order of presenting the two fragrances was randomized. There was widely distributed similar activation in the extended olfactory system by the two fragrances. However, paired t-test indicated that invigorating fragrance was associated with stronger activation in the anterior cingulate, supplementary motor area, bilateral primary visual cortices, middle temporal gyri, and postcentral cortices, which are linked to emotion, memory, imagery, attention and action-oriented processing in addition to olfaction. In contrast, the relaxing fragrance produced significantly weaker activation. The results indicated that olfactory stimuli elicited distinctive patterns of brain activation in multiple systems, suggesting potential for characterizing the neural substrate of sensory, emotional and other brain responses to discrete stimuli. Supported by Quest International Fragrance Company.

361 Poster : Human Olfaction: Psychophysics

OLFACTORY EFFECTS OF ENVIRONMENTAL MALODOR

Bell J.[1], Hirsch A.R.[2] *[1]Rush Presbyterian St. Luke's Medical Center, Chicago, IL; [2]Psychiatry, Rush Presbyterian St. Luke's Medical Center, Chicago, Illinois*

OBJECTIVE: Analysis of health effects of odors emanating from a rubber processing plant on local residents. METHODS: Twenty-five nearby residents, 17 female and 8 male, who complained of malodor were studied. Questionnaires, including demographics, odor frequency, quality, intensity, and health assessments were completed. Subjects underwent physician evaluations, Thiophane equivalency scaling of Amoore, and the University of Pennsylvania Smell Identification Test (UPSIT) for olfactory ability. RESULTS: Sixty-five percent (16) felt the odor affected their health. Headaches, burning nose, and burning eyes were the most frequent symptoms occurring 64% (16), 53% (13), and 48% (12) of the time, respectively. The usual average Thiophane equivalency level was 43 decismels, with an average maximum level of 51 decismels. The UPSIT, corrected fro age and gender, revealed 40% (10) of the subjects were microsmic. When corrected for possible confounding origins of smell loss, 31% (8) were microsmic as compared to a 2% frequency in the general population. DISCUSSION: Clinical symptoms and the Thiophane equivalency test demonstrated that chemical levels frequently exceeded the trigeminal threshold. Intermittent exposure to trigeminal levels of chemicals superimposed upon chronic malodor may have caused the olfactory toxicity. An assessment of health effects at other locations where malodor is produced is warranted. The above study was funded by the Attorney General´s Office of the State of Illinois.

362 Poster : Human Olfaction: Psychophysics

ODOR SIMILARITY INDEX USING BINARY MIXTURE

Chida M.[1], Sone Y.[1], Tamura H.[2], Nagata H.[1], Shikata H.[1] *[1]Tobacco Science Research Center, Japan Tobacco Inc., Yokohama, Japan; [2]Bioresorce Science, Kagawa University, Kita-Gun,Kagawa, Japan*

Research on the odor qualities with verbal description often faces the difficulty to discriminate the odor characters of several different odorants. This difficulty would occur especially when we used abstract descriptors in sensory evaluation, because the understanding for such descriptions might not be always common among subjects. To overcome this difficulty, we tried to establish the methodology that can evaluate the odor qualities without verbal description. Our idea is based on the estimation of relative similarity of odor qualities, which is determined by the discrimination between two odor qualities. We prepared for odorants (isovaleric acid: A, methional: B, β-damascenone: C, skatol: D) which are different from each other by verbal description, and one odorant (3-methyl pentanoic acid: A´) which is similar to odorant A. The intensity of each odorant was matched as "moderate" on Labeled Magnitude Scale. The procedure to measure similarity index between odorant A and B was follows; using an olfactometer, we initially presented subjects odorant A as the "base" odorant. Then we allow subjects to add odorant B (the " additive" odorant) gradually to the base odor by operating the olfactometer until they could distinguish a difference of odor quality between the base odorant (A) and binary mixture (A+B). We could calculate odor similarity index for A vs. B as a ratio of the concentration of odorant B at discrimination to its concentration at "moderate", as well as the pair like A vs. C, A vs. D and so on. As a result, we could show the matrix of odor similarity indices for any pairs of five odorants, in accordance with the verbal description of them.

363 Poster : Human Olfaction: Psychophysics

OLFACTORY PERFORMANCE AND HEALTH STATUS: AN EUROPEAN SURVEY

Rouby c.[1], Thomas-Danguin T.[1] [1]*Universite Lyon1, Lyon, France*

The European Test of Olfactory Capabilities (ETOC) is a self-administrated and short test (needing less than 20 minutes); it has been validated in five European countries (Germany, Denmark, France, Finland and Sweden) on more than 1100 healthy European subjects from 20 to 97 years of age (61% women, 54.618.3 years old). This test is very sensitive (i) to gender differences and (ii) to the decline of olfactory performance along healthy aging This test has also been validated for test-retest reliability. 340 "free living" subjects from 20 to 97 years of age (59.7% women ; 52.2 ± 21.7) filled a questionnaire to record their health status (20 questions). On the basis of the scores obtained at the ETOC, subjects from the panel were divided into two groups : (i) a Low Olfaction Group (LOG) including 88 subjects with low scores (>1 detection error and/or >4 identification errors) and a Normal olfaction Group (NG) with the remaining 252 subjects who obtained higher scores. We asked questions about 11 common health problems. We compared the distribution of people from the low olfaction group and the normal olfaction group. We did not find any difference between the two distributions for people who declared having cholesterol problems, thyroid problems and quite surprisingly ENT problems. But people suffering from respiratory, cardiac, digestive, urinary, muscular problems, as well as diabetes, insomnia and nervous breakdown problems were found in a higher proportion (2 test, $p< 0.05$) in the Low Olfaction Group . Results of our European survey indicate that there are some links between olfactory capabilities and health status, smoking habits, food habits and food choice.

364 Poster : Human Olfaction: Psychophysics

SNIFFING DURING FORMATION OF OLFACTORY IMAGERY

Bremner E.[1], Johnson B.N.[2], Mainland J.D.[1], Young N.[1], Aframian D.[1], Bensafi M.[1], Sobel n.[1] [1]*Neuroscience, University of California, Berkeley, Berkeley, California;* [2]*Bioengineering, University of California, Berkeley, Berkeley, California*

External stimulation can often impinge on internal self-generated representations. For example, when trying to construct mental images of a scene, humans often close their eyes. This blocking of external visual input facilitates generation of an internal visual scene. By contrast, it was our impression that the opposite is true for olfaction, namely, that when trying to form odor imagery, humans take a sniff. To test this we set out to record respiration in 30 subjects during generation of visual, auditory and olfactory imagery. Subjects were cued to generate internal representations of 10 pleasant and 10 unpleasant olfactory, visual, or auditory scenes with ISI of 45 s. For example: "Imagine hearing a scream"; "imagine smelling a skunk"; "imagine seeing a mutilated body." Subjects pressed a button when they had judged themselves to have obtained a mental image. During the task we measured GSR, EMG, and nasal airflow. Subjects were unaware of airflow measurement. In the 6 subjects studied so far, latency to imagery was slowest for olfaction ($p < .05$) and the self-judged quality of the image was lowest. These results agree with previous findings indicating that olfactory imagery is harder to conjure than imagery in other modalities. As predicted, there was a pronounced trend towards increased airflow (a sniff) following the instruction for olfactory, but not for visual or auditory imagery. Data will be presented from the projected cohort of 30, and the significance of this finding regarding cortical processing of olfaction will be discussed. Funding: NIH NIDCD and SOSI

365 Poster : Human Olfaction: Psychophysics

SUPRA-THRESHOLD EVALUATIONS OF OLFACTORY STIMULI BY PROP CLASSIFIED INDIVIDUALS

Kirkmeyer S.V.[1], Tepper B.J.[2] [1]*International Flavors & Fragrances, Inc., Dayton, NJ;* [2]*Food Science, Rutgers University, New Brunswick, NJ*

Sensitivity to the bitterness of PROP (6-n-propylthiouracil) correlates with heightened perception of oral stimuli that have a trigeminal component including capsaicin, ethanol and fat. Greater sensitivity to these compounds is related to greater papillae density on the tongue and more trigeminal input. A specific anosmia has been reported for diacetyl (Lawless et al., 1994) a buttery aroma that also stimulates nasal irritation. Yackinous and Guinard (2001) showed that PROP tasters had a lower olfactory threshold for this compound. Taken together, these data suggest that olfactory sensitivity to diacetyl may differ between PROP taster groups, possibly arising from irritation in the nose. The objective of this study was to investigate supra-threshold ratings of aroma and nasal irritation for diacetyl, ethanol and phenylethylacetate (PEA, rose aroma; a pure olfactory stimulant used as a control) among PROP classified individuals. Fourteen Nontasters and 17 Supertasters evaluated the perceived intensity of diacetyl (1.28, 2.56 and 5.12 mg/l), PEA (50, 100 and 200 mg/l) and ethanol (6.25, 12.5 and 25%) using 15cm visual-analog scales. Compared to Nontasters, Supertasters perceived greater aroma and nasal irritation from diacetyl and greater aroma intensity from PEA (taster main effects; $p<0.05$). No PROP-taster effect was observed for ethanol. These initial findings indicate that Supertasters perceive greater intensity from some olfactory stimuli and both aroma and nasal irritation contribute to these differences. These findings suggest a possible role for PROP status in flavor perception.

366 Poster : Human Olfaction: Psychophysics

THE RELATIONSHIP BETWEEN THE NASAL CYCLE AND PREFERRED HANDEDNESS

Stein E.J.[1], Brzuszkiewicz L.[1], Searleman A.[1], Hornung D.E.[2] [1]*Psychology, St. Lawrence University, Canton, NY;* [2]*Biology, St. Lawrence University, Canton, New York*

The nasal cycle is a pattern where when one nostril is more congested (less open) the other nostril is more decongested (more open) and the nostril that is more open switches periodically. In an effort to explain some of the variability in the nasal cycle, the present study considered the relationship between a person's preferred handedness and the amount of time each nostril was more decongested. Hot wire anemometers mounted at the external naris measured the airflow in the individual nostril every 15 minutes for a 6-hour period in 20 healthy male subjects (11 right-handers and 9 left-handers). Following each flow measurement, subjects reported which nostril they perceived to be more open. The maximum (peak) flow rate recorded from each nostril was used to determine which nostril was more open. Although there was considerable individual variability, on the average, subjects had 4 to 5 changes in nostril dominance during the 6-hour period. Since subjects were correctly able to identify which nostril was dominant only 65% of the time, these results suggest that self-report is not a reliable measure of nostril dominance. However, using peak flow rate as the measure of nostril dominance, for left-handed subjects the left nostril was more open 59% of the time ($p<0.01$) whereas for right-handed subjects the right nostril was more open 59% of the time ($p<0.01$). These results demonstrate for the first time a relationship between the nasal cycle and handedness, and suggest that considering the handedness of subjects might reduce some of the variability evident in most nasal cycle studies.

367 Poster : Human Olfaction: Psychophysics

RATING PROPERTIES OF ODORANTS

Pouliot S.[1], Hudry J.L.[1], Jones-Gotman M.[1] *[1]Montreal Neurological Institute, Canada*

Perception of odors can give rise to subjective judgements. Important interpersonal variations, especially between genders, are observed in responses to odors, with women tending to be superior in several perceptual aspects of olfaction (Brand & Millot, 2001). The main goal of this study was to investigate the relationships among odor properties (intensity, pleasantness, familiarity and arousal) using a new graphic rating scale, and also to ascertain whether men´s and women´s ratings differed. We tested 41 healthy volunteers (26 women) individually. Subjects smelled 131 odors, each subject receiving a different random order. Subjects sniffed each odor for 5 seconds, then rated its perceived intensity, pleasantness, familiarity and arousal on a 1 to 9 graphic scale, after which they attempted to name the odor. We found a strong correlation between intensity and arousal ratings and also between familiarity and arousal ratings. Interestingly, men´s and women´s intensity ratings did not differ but there was a significant gender difference in the more subjective ratings of pleasantness and arousal and for familiarity. Men and women did not differ in the total number of names given to identify the odors nor in the number of inappropriate names given. However, women attributed significantly more absolute-correct or category-correct names. Our finding of a strong correlation between intensity and arousal ratings finds support in Bensafi, et al.´s (2002) similar finding using a different method; we conclude that our new rating scale yields meaningful data and can be used to explore individual and gender differences in olfactory perception. Supported by FCAR, the Savoy Foundation and CIHR.

368 Poster : Human Olfaction: Psychophysics

ODOR QUALITY AND QUANTITY IN CROSS-ADAPTATION

Warren C.B.[1], Schmidt R.[1], Wise P.[1], Polak E.H.[2], Cain W.S.[1] *[1]Chemosensory Perception Lab, Surgery (Otolaryngology), University of California, San Diego, La Jolla, California; [2]Laboratoire de Neurophysiologies Enseignement, Université Paris VI, Paris, France*

The perceived magnitude of an odor depends not only on concentration, but on what a person smelled just before it. In the cross-adaptation paradigm, one odor smelled just after another may smell weaker, to one degree or another, depending upon some as yet unknown commonality between the first and second materials. If the second comprises a mixture of the first and another material, then the second will smell more like the other, i.e., its odor quality will change predictably. To the best of our knowledge, no one has actually measured this change, nor has anyone asked the more generic question of whether the quality of the second will change even when the second does not contain the first, but when the first and second show cross-adaptation of intensity. Three experiments with more than 40 Ss explored the matter, the first after a search for a binary mixture that would mimic a test material (veloutone). The first experiment employed novel psychophysical methodology, a procedure of equivalence, to establish a mixture of linalyl acetate and undecanone, the chosen ingredients, that would come closest to the quality of veloutone. The second experiment established that cross-adaptation of intensity occurred for the relevant combinations of stimuli. The third experiment showed that cross-adaptation can alter odor quality, though not dramatically. In addition to its substantive outcome, the work forges a structure to incorporate variables of quantity and quality into psychophysical investigations.

369 Poster : Human Olfaction: Psychophysics

INDIVIDUAL DIFFERENCES IN HUMAN OLFACTORY COMMUNICATION OF EMOTIONS

Chen D.[1], McClintock M.K.[2] *[1]Psychology, Rice University, Houston, TX; [2]Institute for Mind and Biology, University of Chicago, Chicago, IL*

Previous work of ours demonstrated that sweat collected from different emotional states from the same individual can be distinguished based on their olfactory qualities. This present study examined whether olfactory discrimination of emotional signals may be related to individual differences in understanding and processing emotions in general. Apocrine and eccrine secretions were collected from the underarm and areola areas from a group of men and women when they were under neutral and emotional states of happiness, fear, and sexual arousal. These were in turn evaluated by a group of judges on a series of odor discrimination tasks, the judges' capacity to understand and process emotions assessed using established scales. Individual differences in processing emotions were positively related to olfactory discriminations between some emotions, and negatively related to others. Findings will be discussed in terms of the role of cognition and emotion in processing odor-mediated emotional signals. This work was supported by funding from SOSI, IFF, and a NIMH Merit Award to MKM.

370 Poster : Human Olfaction: Psychophysics

OLFACTORY DIFFERENCES BETWEEN PREGNANT AND NON-PREGNANT WOMEN

Broman D.A.[1], Olofsson J.[1], Olsson M.J.[2], Nordin S.[1] *[1]Department of Psychology, Umeå University, Umeå, Sweden; [2]Department of Psychology, Uppsala University, Uppsala, Sweden*

There are many women who claim to perceive olfactory changes during pregnancy. In the same vein, other changes occur during pregnancy that relate to the chemical senses, such as nasal congestion, food perception, cravings and aversions. Although some of these changes have gained more interest and documentation, the focus on olfaction per se has been limited. The aim of this study was, therefore, to more thoroughly investigate various olfactory functions during pregnancy. Thirty pregnant women (23-38 years, M=30.6) in week 22-24 of pregnancy and a control group of 30 women (24-36 years, M=27.8), who never had been pregnant, were tested with psychophysical and perceptual methods. In the psychophysical part detection thresholds and thresholds for perceived unpleasantness (method of constant stimuli, corrected for false alarms) were determined. Additionally, intensity ratings and unpleasantness ratings (method of magnitude estimation, calibrated for response behavior according to the master scale principle) were conducted. These measurements were done for a set of 8 pyridine concentrations, 20-20000 ppb. The perceptual part included a variety of judgments of a set of 32 odors. The perhaps most interesting findings were differences in thresholds. Pregnant women had higher absolute odor sensitivity compared to controls and appeared to be even more sensitive with respect to odor unpleasantness. Supported by grants from the Swedish Research Council.

371 Poster : Human Olfaction: Psychophysics

A LONGITUDINAL STUDY OF FOOD AVERSIONS AND CRAVINGS IN PREGNANT WOMEN

Nordin S.[1], Broman D.A.[1], Olofsson J.[1], Wulff M.[2] *[1]Department of Psychology, Umeå University, Umeå, Sweden; [2]Department of Obstetrics and Gynecology, Umeå University, Umeå, Sweden*

Previous findings suggesting that food aversions and cravings are common among pregnant women motivated the present longitudinal study of how pregnant women in a broad perspective relate to food and the role of the chemical senses. Pregnant women in pregnancy weeks 16 and 34 and 12 weeks post partum and non-pregnant women with corresponding intervals, responded to the Craving Questionnaire, to questions about food aversions analogous to the Craving Questionnaire, to the Food Neophobia Scale and the Disgust Scale, and to questions about smell and taste perception and nausea. The results showed higher prevalences of hypersensitivity to odors (68%; e.g., cooking odors, spices, coffee) and bitter tastants (16%), parosmia (17%) and phantosmia (13%), abnormal food cravings (28%), food aversions (57%), nausea (80%), and higher scores on food neophobia but not on disgust in pregnancy week 16, and somewhat lower frequencies and scores in week 34, relative to post partum and non-pregnant women. Most pregnant women referred these conditions to their pregnancy (73-96% depending on type of condition). High prevalences of aversions and cravings were also found to be associated with high prevalences of self-reported abnormal smell and taste sensitivity, respectively. These findings suggest that an abnormal relation to food is common in pregnancy and that the chemical senses play an important role in this respect. Supported by grants from the Swedish Research Council.

372 Poster : Human Olfaction: Psychophysics

EFFECTS OF AGE, SEX, AND SIDE OF STIMULATION ON CHEMOSENSORY EVENT-RELATED POTENTIALS

Larsson M.[1], Lundstrom J.N.[2], Frasnelli J.[3], Hummel T.[3] *[1]Dpt of Psychology, Stockholm University, Stockholm, Sweden; [2]Psychology, Uppsala University, Uppsala, Sweden; [3]University of Dresden Medical School, Dresden, Germany*

The aim of this study was to explore effects of age, sex, and stimulation side on chemosensory event-related potentials [ERP]. Further, we investigated the relationships between ERP measures and cognitive performance. 39 younger adults (19 f, 20 m; range 19-33 years), and 28 older adults (14 f, 14 m; range 52-79 years) participated. Subjects underwent olfactory testing (PEA odor threshold, odor discrimination and identification), and psychological tests tapping various cognitive domains (e.g., cognitive speed, working memory). ERPs were recorded in response to either left- or right-sided stimulation with H2S, PEA, and, in younger subjects, also to CO_2. Older subjects had longer ERP peak latencies [L] and smaller amplitudes [A]; this change was more pronounced in females compared to males. Main effects of sex were seen for trigeminal, but not olfactory ERP with larger A observed in women. Identification correlated with all ERP components, while discrimination typically correlated with later ERP peak L; PEA thresholds and ERP were not correlated. Measures of working memory exhibited a higher degree of correlation with later ERP components whereas attentional measures had a similar degree of correlation with all ERP measures. These results indicate specific roles of earlier and later components, respectively, of chemosensory ERPs in the processing of odorous information. Support: Swedish Research Council (F0647/2001) and Deutscher Akademischer Austausch Dienst (A/02/08254) to ML, and the DFG (HU441/2) to TH.

373 Poster : Human Olfaction: Psychophysics

EFFECTS OF STIMULATED NOSTRIL ON OLFACTORY EVENT-RELATED POTENTIALS

Olofsson J.[1], Broman D.A.[1], Gilbert P.E.[2], Nordin S.[1], Dean P.[3], Murphy C.[2] *[1]Department of Psychology, Umeå University, Umeå, Sweden; [2]Department of Surgery, University of California, San Diego School of Medicine, San Diego, CA; [3]Department of Psychology, San Diego State University, San Diego, CA*

Recordings of olfactory event-related potentials (OERPs) are typically conducted using a monorhinal stimulus presentation with no or limited consideration taken to side of stimulated nostril. The aim of the present study was to investigate possible effects of stimulated nostril on OERP amplitudes and latencies. OERPs (P1, N1, P2, N2, and P3) in response to amyl acetate were recorded at Fz, Cz and Pz in young (18-30 years), right-handed, healthy adults, screened for loss in olfactory sensitivity and upper respiratory disease. Nostril sides did not differ in self-reported congestion. The results showed that the P2, P3 and N1P2 (peak-to-peak) amplitudes were larger, and the P2 latency longer when stimulating the right nostril, compared to the left. Judging from these results, the stimulated nostril should be accounted for when OERPs are obtained through monorhinal stimulation, in particular regarding the later components. The results of larger amplitudes can be related to previous findings of a right-nostril advantage for detection and quality discrimination. However, further research is needed to clarify the possible role of laterality in OERP amplitudes and latencies. Supported by NIH grant #DC02064 (CM) and grants from the Swedish Research Council (SN).

374 Poster : Human Olfaction: Psychophysics

OLFACTORY EVOKED POTENTIALS DURING MONORHINAL VERSUS BIRHINAL STIMULATION WITH LINALOOL

Kobal G.[1], Wille C.[1], Mueller C.[1], Kettenmann B.[1] *[1]University of Erlangen-Nuremberg, Erlangen, Germany*

There is evidence that birhinal stimulation increases intensity estimates, but not in a linear fashion. So far, it has not been investigated, how OEPs integrate birhinal stimulation with odorants. In this study, intensity and hedonic estimates, as well as evoked potential amplitudes and latencies were investigated when Linalool was administered in 3 concentrations to only one (right) or to both nostrils. Seven subjects participated in the experiments. Intensity estimates in both modes of stimulation, resulted in a clear - almost linear - dose response curve. As predicted, birhinal stimulation yielded higher intensity estimates. However, the increase in ratings was only approx. 25%. Birhinal stimulation resulted in higher amplitudes in the OEPs as well. When intensity estimates increased, hedonic ratings decreased, i.e. the more intense Linalool was perceived, the less pleasant it became. Similar to intensity, unpleasantness increased as a result of birhinal stimulation. However, the increase in OEP amplitudes was not homogeneous throughout the recording positions. This indicates that an amplitude increase might be due to superposition of activities from different electrical generators that do not process the information of both nostrils uniformly. In conclusion, OEPs behaved similar to subjective estimates only in the central recording position Cz. The right hemispheric sites did not show this effect at the higher concentration indicating a lateralization of odor intensity coding. This research was supported by Firmenich S.A.

375 Poster : Human Olfaction: Psychophysics

INFLUENCES OF ETHANOL INGESTION ON OLFACTORY FUNCTION: SPECIFIC TO ETHANOL ODOR

Doty R.L.[1], Patel S.J.[1], Bollhoefer A.[1] *[1]Smell and Taste Center, University of Pennsylvania, Philadelphia, PA*

The influence of ethanol ingestion on four psychophysical measures of olfactory function was determined in 8 women and 8 men. In this double-blind study, grape juice alone was administered prior to the olfactory tests on one test occasion, and a grape-juice vodka mixture, designed to produce blood levels of ethanol near the legal level of intoxication, was administered prior to the tests on the other occasion. The test measures, whose order of presentation was counterbalanced among subjects using Latin squares, were the University of Pennsylvania Smell Identification Test, an odor discrimination/memory test, an ethanol odor detection threshold test, and a phenyl ethyl alcohol odor detection threshold test. Breath alcohol analysis was determined every 15 minutes throughout the 2 1/2 hour test sessions, which were counterbalanced in order across subjects and separated from one another by a minimum of a week. Ethanol threshold values were markedly elevated in all subjects on the ethanol test occasion relative to the control test occasion. No other olfactory measure differed between the two test occasions. These findings indicate that alcohol ingestion markedly alters sensitivity to ethanol, but does not influence other common measures of olfactory function, and provide empirical support for the "cocktail party alcohol effect;" namely, that persons who drink alcohol have more difficulty than those who do not in discerning alcohol on the breath of others.

376 Slide : Functional Organization of Olfactory Systems

PHEROMONE CODING BY THE MAMMALIAN MAIN OLFACTORY EPITHELIUM

Leinders-Zufall T.[1], Ziesmann J.[1], Puche A.C.[1], Bock R.[1], Ma W.[2], Novotny M.V.[2], Zufall F.[1] *[1]Anatomy & Neurobiology, University of Maryland, Baltimore; [2]Institute for Pheromone Research and Dept. of Chemistry, Indiana University Bloomington, IN*

Most mammalian species have two distinct sensory organs for the detection of chemical cues, the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). The prevailing view is that the MOE detects volatile odorants that provide information about the world at large whereas the VNO is specialized for the detection of pheromones. Here we report that pheromones that are high potency ligands for neurons in the VNO are also detected by the MOE. Thus, in mammals the VNO is not the exclusive site of pheromone detection. Using a new mouse MOE slice preparation in combination with electrophysiological and optical imaging techniques, we show that subpopulations of MOE sensory neurons are extremely sensitive pheromone detectors, with detection thresholds in the picomolar range. Individual sensory neurons can respond to multiple pheromones, suggesting that pheromone discrimination in the MOE is based on a combinatorial coding scheme. The spatial distribution of pheromone-responsive neurons indicates the presence of preferential activation zones, broadly coincident with olfactory receptor gene expression zones. These results highlight the dramatically different coding strategies underlying discrimination of the same pheromone ligands in the MOE vs. VNO. Supported by NIDCD (TL-Z, MVN, and FZ).

377 Slide : Functional Organization of Olfactory Systems

MOLECULAR ORGANIZATION OF THE MOUSE SEPTAL ORGAN

Ma M.[1], Iwema C.L.[2], Liu J.[3], Greer C.A.[2], Shepherd G.M.[3] *[1]Neuroscience, University of Pennsylvania, Philadelphia, PA; [2]Neurosurgery, Yale University, New Haven, CT; [3]Neurobiology, Yale University, New Haven, CT*

The mouse septal organ is a small island of olfactory neuroepithelium located bilaterally at the ventral base of the nasal septum. It constitutes approximately 1% of the olfactory epithelium. Previously we have demonstrated that cAMP is the major second messenger mediating olfactory signal transduction of the receptor cells in this area. However, its olfactory receptors have not been studied. Because the majority of the septal organ sensory neurons express olfactory-specific G-protein, we hypothesize that these neurons also express G-protein coupled olfactory receptors. We cloned ~40 olfactory receptor genes from the septal organ following a standard RT-PCR protocol by using degenerate primers, which were based on the conserved regions of all the mouse olfactory receptor genes. These genes do not form a distinct subfamily. They are located in multiple clusters on different chromosomes. In situ hybridization showed that many of these receptor genes are shared with the main olfactory epithelium. Further molecular and functional analysis of the septal organ should provide new insights into the organization of the mammalian olfactory system and the unique function(s) that this enigmatic organ may serve. This work was supported by grants to M.M. from NIDCD/NIH (DC05127) and the Whitehall Foundation, by NIDCD grants (DC00210 and DC03887) to C.A.G., and by grants to G.M.S. from NIDCD (DC00086); NIDCD, NIA, NASA and NIMH (Human Brain Project); and ARO (MURI).

378 Slide : Functional Organization of Olfactory Systems

MAPPING ODOR RECEPTORS TO NEURONS

Goldman A.L.[1], Lessing D.[1], Van Der Goes Van Naters W.[1], Warr C.[2], De Bruyne M.[3], Carlson J.R.[1] *[1]Molecular, Cellular, Developmental Biology, Yale University, New Haven, CT; [2]School of Biological Sciences, Monash University, Melbourne, Australia; [3]Freie Universitaet Berlin, Berlin, Germany*

In order to define the ligand specificity of individual odorant receptors (Ors) and to study the functional organization of the olfactory system, we are mapping Ors to ORNs. In the maxillary palp of *Drosophila melanogaster*, there are 6 ORN classes, defined by their electrophysiological response to odors. These 6 cell classes adhere to a strict pairing rule, e.g. a pb1 sensillum contains a pb1A cell paired with a pb1B cell. Four of these cell classes are altered or missing in the *acj6* mutant, in which Or gene expression is also altered. We labeled ORNs using Or promoters to drive the expression of GFP. We then recorded electrophysiologically from these labelled sensilla in live flies to determine their odor sensitivity, and to map the Or to a specific sensillar type. mRNA in situ hybridizations and double labeling experiments were done in wild type flies and in *acj6* mutants to map Ors to specific ORN classes. Probes for particular pairs of Or genes were found to label pairs of adjacent cells that cohabit specific types of sensilla. This analysis has allowed us to map individual receptors to the neurons that express them. Some of the receptors appear narrowly tuned, but the data support a model in which an odor can elicit a response from two neurons via different receptors. Supported by: R01 DC 04729 and DC 02174

379 Slide : Functional Organization of Olfactory Systems

THE GR21A RECEPTOR AND CARBON DIOXIDE PERCEPTION IN *DROSOPHILA*

De Bruyne M.[1], Schwarz S.[1], Wendt M.[1], Regnery B.[1], Galizia C.G.[1], Fiala A.[2], Diegelmann S.[2], Buchner E.[2], Carlson J.R.[3] *[1]Freie Universitaet Berlin, Berlin, Germany; [2]Theodor-Boveri-Institut, Wuerzburg, Germany; [3]Yale University, New Haven, CT*

Olfaction in Drosophila most likely involves combinatorial coding. However, certain odors may be encoded via `labelled lines'. 22 classes of ORNs with different response spectra have been described. Each class expresses a member of a family of olfactory receptors (OR) but a few gustatory receptors (GR) are also expressed in antenna, one of which is Gr21a. We recorded from ab1C neurons that respond exclusively to CO_2 with thresholds around ambient concentrations (0,03%). We then used the GAL4-UAS system to drive several reporter genes under control of the Gr21a promoter. The expression pattern of GFP suggests that Gr21a is expressed in ab1 sensilla. Gr21a-driven expression of the cell-death gene rpr deletes most GFP positive cells. Electroantennograms of such flies show a marked reduction in responses to CO_2 but not to other odorants. We also expressed calcium sensitive cameleon in ab1C neurons. In optical recordings on transgenic flies we measured a dose-dependent activation by CO_2 but not by other odorants. In addition, the V glomerulus was labeled and showed similar responses. We conclude that ab1C neurons express the gustatory receptor GR21a. It is noteworthy that the Anopheles gambiae Gr22 receptor shows high homology with Drosophila Gr21a. Mosquitoes are known to use CO_2 as a cue in host finding. In a simple orientation assay, Drosophila avoids a wide range of CO_2 concentrations. The response reaches saturation at a dose well below anesthetic levels that generates 50 spikes/s from ab1C neurons. Such a response may serve to avoid noxious levels of CO_2 present in fermenting fruits. Supported by the DFG, Sonderforschungsbereich 515

380 Slide : Functional Organization of Olfactory Systems

HIGH THROUGHPUT MICROARRAY DETECTION OF OLFACTORY RECEPTOR GENE EXPRESSION

Zhang X.[1], Rogers M.[1], Zhang X.[1], Zou D.[1], Liu J.[2], Ma M.[2], Shepherd G.M.[2], Firestein S.[1] *[1]Biological Sciences, Columbia University, New York, NY; [2]Neurobiology, Yale University, New Haven, Connecticut*

Genomic study in the draft mouse genome has revealed more than 1000 putative olfactory receptor (OR) genes and ~150 vomersonasal receptor V1R genes. To study the expression profile of these genes, we have designed a custom made high-density oligonucleotide array for mouse OR and V1R genes. This has enabled detection of a large number of the receptor genes and monitoring of receptor gene expression across the entire olfactory epithelium, as well as in various tissues, developmental stages, and genetic backgrounds. Specific expression in the olfactory epithelium has been demonstrated for about 750 OR genes, providing strong evidence that those genes designated as ORs, based only on genomic sequences, are indeed likely to function in the appropriate olfactory tissue. Similarly, specific expression in the vomeronasal organ has been shown for a number of V1R genes. Monitoring spatially separate zones of the olfactory epithelium revealed a heterogeneous expression pattern for over 400 OR genes. Nearly half of the OR genes, including all of the "fish-like" Class I ORs, were expressed in the most dorsal epithelial zone. Interestingly, OR genes expressed in different zones of the epithelium were also segregated on the chromosomes. This correlation between genomic location and spatial expression provides new insights into the regulation of this large family of genes. Supported by NIDCD, Human Brain Project, and MURI (GMS and MM)

381 Slide : Functional Organization of Olfactory Systems

PROCESSING OF ODOR BLENDS IN THE INSECT ANTENNAL LOBE

Kleineidam C.[1], Vickers N.J.[1], Linn C.[2] *[1]Biology, University of Utah, Salt Lake City, Utah; [2]Entomology, Cornell University, Geneva, New York*

Processing within the antennal lobe modifies the final representation of odors in the assembly code carried by projection neurons to higher brain regions. The gross morphology of the insect antennal lobe is analogous to the olfactory bulb of vertebrates where lateral inhibition between neighboring glomeruli via mitral cells has been found. In contrast to the olfactory bulb, no clear morphological correlate to mediate lateral inhibition has been found in the antennal lobe. We examined the mutual influence of neighboring glomeruli by recording intracellularly from projection neurons of the moth *Heliothis virescens* during single odor and binary mixture stimulation. We found that the responses of component-specific projection neurons were reduced when the primary component was presented as a mixture with another pheromone compound (to ~73%, n=43). The level of inhibition was constant over a wide range of concentrations of the second compound (2-2000x the 1° compound). The inhibition was dependent on the distance between the two glomeruli where the two pheromones of the binary mixture were primarily represented. Inhibition was weaker as the distance between glomeruli increased (response reduced to ~58% in adjacent glomeruli vs. ~83% in separated glomeruli, n=9). These results show that odor information processing is functionally similar in the antennal lobe and the olfactory bulb. However, the mechanism of lateral inhibition differs. Lateral inhibition may be mediated by either antennal lobe local neurons or NO release by receptor neurons. NIH 1 R55 DC04443-01 to CL.

382 Slide : Functional Organization of Olfactory Systems

FUNCTIONAL ORGANIZATION OF INPUT TO THE MOUSE OLFACTORY BULB GLOMERULUS VISUALIZED WITH TWO-PHOTON CALCIUM IMAGING

Wachowiak M.[1], Friedrich R.W.[2] *[1]Dept. Biology, Boston University, Boston, MA; [2]Dept. Biomedical Optics, Max-Planck-Institute for Medical Research, Heidelberg, Germany*

The glomerulus receives sensory input from olfactory receptor neurons (ORNs) expressing the same odorant receptor and is thought to act as a functional unit in olfactory processing. However, whether the glomerulus is functionally homogeneous has been difficult to address. We used two-photon imaging, in combination with selective loading of ORNs with calcium-sensitive dye, to image odorant-evoked receptor neuron input within single glomeruli at high spatial and temporal resolution. Evoked calcium signals were distributed heterogeneously, with 'hot spots' of large-amplitude signal presumably representing densely packed nerve terminals. However, the hot spots appeared to be distributed with equal probability throughout the glomerular volume. Thus, we did not find any evidence for functional subdivisions within the glomerulus, at least at the level of receptor neuron input. Structurally distinct odorants presented at similar effective concentrations elicited similar spatial patterns of calcium signal within the same glomerulus. While the time course of the odorant-evoked signal was concentration-dependent, at a given concentration it was similar throughout the glomerulus, except for occasional small differences in response latency. Thus, with few exceptions, receptor neuron input to a glomerulus appears to be spatially heterogeneous but functionally uniform. These results support the hypothesis that the glomerulus acts as a functional unit and integrates sensory input from idiotypic ORNs. Supported by the Max Planck Society, NIH DC00378 and DC05259.

383 Slide : Functional Organization of Olfactory Systems

GLOBAL REPRESENTATION OF CHEMICAL STRUCTURES IN THE MOUSE OLFACTORY BULB REVEALED BY FMRI

Xu F.[1], Liu* N.[1], Kida# I.[1], Rothman# R.L.[1], Hyder# F.[1], Shepherd* G.M.[1] [1]*Neurobiology* and Diagnostic Radiology#, Yale University, New Haven, CT*

It is generally accepted that odor information is represented as activity patterns across the glomerular layer in the olfactory bulb. However, how the chemical structures are represented by these patterns is poorly understood. To reveal the encoding principles, the global activity patterns in the same mouse elicited by odorants with different specific structural relationships were obtained by functional MRI. The medial and lateral regions of the olfactory bulb were generally the most activated, with usually asymmetrical levels of activity. For odorants with different functional groups but same carbon number, the most highly activated areas in the glomerular layer rarely overlapped, so that the patterns were distinctly different from each other. In contrast, for the homologous aliphatic series, the highly activated areas were significantly more likely to be shared by all members, the patterns overlapping but with different `family-signatures´. Moderately active areas were more sensitive to the length of the carbon chain, producing more subtle features of individual members and different changing trends among homologues. The results suggest that chemical structures are represented hierarchically: functional group is a primary factor determing the main pattern features of an odorant `family´, whereas carbon chain is a secondary factor carrying information about more detailed characteristics of a specific member of a family. The research is supported by NIH grants RO1-DC00086 (GMS), RO1-MH52550-5 (GMS), NS-37203 (FH), and NS-32126 (DLR), and a DOD MRUI grant (GMS).

384 Poster : Olfactory Receptor Cells

EFFECT OF TAURINE SUPPRESSION ON THE OLFACTORY SYSTEM OF THE MOUSE

Witt M.[1], Roth C.[1], Hummel T.[2], Warskulat U.[3], Bidmon H.[4] *[1]Department of Anatomy, University of Technology, Dresden, Germany; [2]University of Dresden Medical School, Dresden, Germany; [3]Clinic for Gastroenterology, Hepatology and Infectiology, Heinrich Heine University, Düsseldorf, Germany; [4]C & O Institute for Brain Research, Heinrich Heine University, Düsseldorf, Germany*

Taurine (2-aminoethansulfonic acid) occurs in high concentrations in animals and humans. In the cerebellum, retina, and olfactory system (OS) its concentration exceeds even that of glutamate. In taurine transporter knockout mice, degeneration of the retina as well as developmental retardation of the cerebellum has been documented. The present study aimed to investigate effects of taurine suppression on both, olfactory epithelium (OE) and olfactory bulb (OB). Experiments were made in mice, in which the taurine transporter gene had been knocked out and in wildtype variants. We used immunohistochemical procedures for detecting specific olfactory and more general neuronal markers (OMP, PGP 9.5), glia markers (GFAP, S-100), proliferation markers (PCNA, Ki67), apoptotic marker (caspase-3), or stress markers (heat shock protein 25: HSP25). The results indicated that taurine transporter knockout mice accumulated almost no taurine IR in supporting cells of the OE and in interneurons of the external plexiform layer of the OB. However, expression of most functional markers including OMP, GFAP, and caspase-3 was not different between widltype variants and knockout mice. Preliminary quantification of proliferating cell numbers indicated a decrease of dividing supporting cells in knockout mice. Moreover, expression of HSP25 supporting cells of knockout mice appeared to be upregulated compared to wildtype controls. In summary, our preliminary results in the OS indicate that taurine transporter knock out mice seem to be able to compensate in part taurine deficiency by other mechanisms, e.g., heat shock proteins which are known to contribute to a reduction of oxidative stress related sequelae.

385 Poster : Olfactory Receptor Cells

NEW INTRACELLULAR STAINING TECHNIQUE APPLIED TO *DROSOPHILA* NEURONS

Wada S.[1], Kanzaki R.[2] *[1]Life and Environmental Sciences, University of Tsukuba, Tsukuba-shi, Ibaraki, Japan; [2]biologycal science, University of Tsukuba, Tsukuba-shi, Ibaraki, Japan*

The fruit fly, *Drosophila melanogaster*, has been of particular interest for the study of olfaction because of its suitability for genetic manipulation, the recent availability of a complete genome sequence, and its ability to learn in simple olfactory-based associative learning paradigms. Using genetic labeling of projection neurons, morphological analyses have been done. However, genetic labeling of neurons is random staining. In order to gain a better understanding of the morphology of *Drosophila* neurons we developed an intracellular staining technique by which we can directory insert a glass electrode filled with Lucifer Yellow into the targeted cell body of a *Drosophila* neuron. We succeeded in staining many antennal lobe(AL) neurons, including local interneurons(LNs), and projection neurons(PNs) and some other neurons. The LNs arborize throughout in the whole AL, and the PNs arborize in the single glomeruli and project to the protocerebrum through the IACT. In the future we will analyze in more detail the neural circuits of *Drosophila* using Ca indicators and intracellular recording techniques.

386 Poster : Olfactory Receptor Cells

DETECTING CHANGES OF OLFACTORY NEURON ACTIVITY

Blejec A.[1] *[1]Dept. of Invertebrate Physiology, National Institute of Biology, Ljubljana, Slovenia*

Spontaneously active olfactory receptor neuron (ORN) responds to chemical stimulation by change of action potential firing rate. During the response, the firing rate can be higher (excitation) or lower (suppression) than spontaneous firing rate. For classification of ORN responses a new method, based on analysis of the cumulative distribution function (CDF) slopes and named "Cumulative Slope Analysis" (CSA), is proposed. The CDF slope at given time represents the firing rate of events at that time. Due to stochastic nature of ORN spontaneous activity the firing rate is characterized with empirical sampling distribution of local slopes. The percentiles of this distribution are used to gauge the extent of the slope changes after stimulation and to detect and classify changes as responses if they are large enough. To control the percentage of false positive response detection α (Type I error) CSA response classification algorithm level of response detection was tuned via extensive computer simulation method. For different percentages α, series of sequences of uniformly distributed events was simulated and analyzed with CSA using different response detection level tuning factors. The optimal tuning factor was used in similar way for assessment of percentage of false negative classifications (Type II error) in which we simulated series of sequences with known levels of change in firing rate. The tuned CSA enables detection of significant changes in firing rate of spontaneously active ORNs and is well suited for analysis of a large number of single unit recordings. This research was supported by grants MSZS J1-3366-0105-02 and SLO-US-2002/18.

387 Poster : Olfactory Receptor Cells

SHORT-TERM CULTURE OF *CAENORHABDITIS ELEGANS* CHEMOSENSORY NEURONS

Nickell T.[1], Kleene N.K.[2], Kleene S.J.[2] [1]*University of Cincinnati College of Medicine, Cincinnati, OH;* [2]*Cell Biology, Neurobiology & Anatomy, University of Cincinnati, Cincinnati, Ohio*

Dissociated cells of C. elegans embryos can develop in tissue culture conditions. Neurons in these cultures exhibit at least some of their adult phenotypic characteristics. It is impossible, however, to be certain to what extent the functional characteristics of these neurons resemble the properties of the neurons in vivo. We have developed a method for short-term culture of adult C. elegans neurons. Worms were transferred to a cooled plate covered with Leibovitz's L-15 medium supplemented with sucrose to bring the osmolality to 340 mOsm. The worms were then cut twice with a #11 scalpel blade: once caudally to relieve internal pressure and then just behind the pharynx to allow exposure of the nerve ring. Individual heads were then transferred to a chamber filled with Leibovitz's L-15 medium supplemented with Fetal Calf Serum and sucrose to bring the total osmolality to 340 mOsm. The GFP-labeled chemosensory neuron AWA maintained characteristic morphology up to 48 hours. Contraction of the body wall muscles and cuticle continued during the culture period so that neurons became progressively more exposed. Dendrites (responsible for chemosensory transduction) of AWA neurons with exposed somata retained a normal morphology and appeared to maintain contact with the amphid sensilla. This contrasts with our previous physiological preparation, in which recordings were done on worm heads in saline shortly after dissection. In these, the dendrite appears broken or beaded within 2-3 hours after cutting. This culture system offers a new and possibly more efficient approach to the study of C. elegans physiology. We are presently investigating the electrophysiological properties of these cultured neurons.

388 Poster : Olfactory Receptor Cells

THE USE OF FRESH POSTMORTEM HUMAN PERIPHERAL OLFACTORY TISSUE FOR PHYSIOLOGIC STUDY

Murrow B.[1], Walton K.[1], Michaels R.[1], Jafek B.[1], Leonard S.[1], Restrepo D.[1] [1]*UCHSC, Denver, CO*

Fresh postmortem human tissue shows promise for the study of peripheral olfactory physiology, including that of olfactory epithelium and olfactory bulbs. Isolated cells from the olfactory epithelium can retain morphologic characteristics of olfactory receptor cells. These cells can exhibit physiologic properties consistent with what is known of human olfactory receptors isolated from live human biopsies. In particular, the postmortem cells can exhibit voltage-gated ionic channels and an electrical response to odorants. EOG recordings show odorant-induced responses, which are selective to specific odorants, dose-dependant, adaptable, and blocked by Ba. Interestingly, some cells isolated at 30 hours postmortem are still responsive to chemical stimuli. Similarly, mouse olfactory receptor cells can be responsive to IBMX at long postmortem harvest times. Preliminary experiments have also examined the use of fresh postmortem olfactory bulb tissue for study. Cells can be isolated and reveal Ca changes to depolarization by application of KCl. Attempts at culturing this tissue has yielded differentiated cells that are currently undergoing identification of cell types by immunohistochemistry and physiologic study by Ca imaging. Overall, continued studies on fresh postmortem human olfactory tissue reveals a viable approach to study olfactory physiology in humans. It eliminates the inherent potential risks associated with obtaining biopsy tissue from live patients and also provides a greater amount of tissue. Furthermore, this system is the only way of obtaining human olfactory tissue located intracranially.

389 Poster : Olfactory Receptor Cells

INHIBITION OF HEAT SHOCK PROTEIN INDUCTION IN MOUSE OLFACTORY EPITHELIUM BY IN VIVO ADMINISTRATION OF PURINERGIC RECEPTOR ANTAGONISTS

Hegg C.C.[1], Davis K.[1], Lucero M.T.[1] [1]*Physiology, University of Utah, Salt Lake City, Utah*

Heat shock proteins (HSPs) accumulate in cells exposed to a variety of physiological and environmental factors such as heat shock, oxidative stress, and odorants. Ischemic, stressed, and injured cells release ATP in millimolar amounts; moreover, intracellular ATP significantly decreases when the olfactory epithelium (OE) is damaged by noxious fumes, presumably through release by injured cells (Kilgour JD et al. *Toxicol* 145, 39-49, 2000). Our hypothesis is that noxious stimulation (in this case, strong odor) evokes the release of extracellular ATP that induces the expression of HSPs via purinergic receptors. As previously shown in rats (Carr VM et al. J Comp Neurol 432, 425-439 2001), *in vivo* odorant exposure (0.02% heptanal, 3-6 hours) led to induction of HSP25 in sustentacular cells in Swiss Webster mouse OE. We investigated whether administration of purinergic receptor antagonists suramin and pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) can block the induction of HSP expression. Mice were injected with 100 µmoles/kg suramin + PPADS and exposed to 0.02% heptanal for 3-6 hours. Purinergic receptor antagonists reduced the expression of HSP25 in sustentacular cells. The OE is constantly exposed to airborne pollutants and microbes, consequently, it continuously regenerates. ATP released by acutely injured cells could act as an early signal of cell damage, evoking HSP expression and stimulating regeneration due to the mitogenic and growth-promoting effects of purinergic receptor activation. This research was supported by NIH NIDCD DC04953.

390 Poster : Histological Profiles in Peripheral Gustatory Systems

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF PALATE TASTE BUDS IN MICE

Stone L.M.[1], Baker L.S.[1], Margolskee R.F.[2], Kinnamon S.C.[1] [1]*Biomedical Sciences, Colorado State University, Fort Collins, Co;* [2]*Medicine, Mount Sinai School of Medicine, New York, New York*

Palate taste buds respond well to sweet and umami compounds and express α-gustducin, a G protein subunit implicated in both bitter and sweet transduction. Circumvallate taste buds respond well to bitter stimuli and like palate taste buds, express α-gustducin. It is presently unclear whether the patterns of α-gustducin expression differ between rodent palate and circumvallate taste buds. The goal of this study was to determine whether α-gustducin is expressed in a similar subpopulation of taste cells in both areas. To do this, we examined the expression of α-gustducin promoter-driven GFP in transgenic mice relative to various immunocytochemical markers in palate and circumvallate taste buds. Our results show that like circumvallate taste buds, α-gustducin and PLCß2 are co-expressed in a subpopulation of palate taste cells. Serotonin immunoreactivity is not found in cells expressing α-gustducin, similar to the expression profile in circumvallate taste buds. However, the numbers of cells expressing serotonin immunoreactivity are lower in palate taste buds relative to circumvallate buds. Most striking, immunoreactivity for the putative sweet/umami receptor subunit T1r3 is co-expressed with α-gustducin in a subpopulation of palate taste cells. In circumvallate taste buds there is little co-expression of T1r3 and α-gustducin . In summary, our results indicate that the immunocytochemical profile of palate taste buds differs from that of circumvallate taste buds, and suggests that T1r3 receptors may activate α-gustducin in the palate. Supported by DC00244 to S.C.K. and DC03155 to R.F.M.

391 Poster : Histological Profiles in Peripheral Gustatory Systems

MECHANICAL STIMULATION RELEASES SEROTONIN FROM RAT TASTE BUDS

Plonsky I.[1], Baur J.E.[2], Pereira E.[3], Roper S.D.[3] *[1]Lab. of Bioinformatics and Motion Control, Institute for Information Transmission Problems, Moscow, Russia; [2]Department of Chemistry, Illinois State University, Normal, IL; [3]Dept. of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL*

The function of taste buds (TBs) is not limited to reception of gustatory stimuli. Mechanosensitivity in TBs has been reported in numerous studies (e.g., Bradley et al 1983; Matsuo et al 1995; Ogawa et al 1997). To date, the release of neurotransmitters in TBs has never been measured directly for any type of physiological stimuli. We used an electrochemical method, fast scan voltammetry with carbon fiber electrodes (CFEs), to search for such release in isolated rat TBs evoked by mechanical and chemical stimulation. CFEs were tested with 2 μM serotonin, 10 μM dopamine, and 10 μM norepinephrine. TBs were preincubated in medium containing 2 mM serotonin or its precursor 5-hydroxytryptophan. Under these conditions, serotonin could be detected in TB cells with immunochemistry. Mechanical stimuli (gentle taps) applied to TBs with the recording electrode induced a substantial discharge of serotonin. The response was not dependent on Ca^{2+} influx because it could be observed in Ca-free medium. TBs that were not preincubated with indolamines did not release neurotransmitters. The tastants cycloheximide and sucrose that are known to increase intracellular Ca^{2+} in some TB cells failed to evoke the release of serotonin. These data imply that serotonin might participate in mechanoreception in TBs. Supported in part by NIDCD/NIH grant 1PO1 DC00244.

392 Poster : Histological Profiles in Peripheral Gustatory Systems

RADIATION INDUCED CHANGES IN HISTOLOGY AND BEHAVIOR IN THE SR90 TASTE LOSS MODEL

Nelson G.[1], Ferraro F.[2], Coonfield D.[2], Kiefer S.[2] *[1]Neurobiology, University of Alabama at Birmingham, Birmingham, AL; [2]Psychology, Kansas State University, Manhattan, KS*

Taste loss and alterations in food perception are common problems resulting from head and neck radiation therapy. The literature suggests more taste alterations than can be explained by receptor loss from stem cell damage. The Sr90 taste loss model allows the correlation of altered taste with alterations in morphology. Sprague Dawley rats received a 0 (control), 6, or 12 cGy dose of radiation to the dorsal tongue via the tongue irradiator and were fitted with an intraoral cannula for the taste reactivity test. Rats were tested for taste responsiveness (ingestive and aversive) with NaCl and QHCl on days 5 and 15 post-irradiation. Animals were sacrificed on day 17 and tissue prepared for light microscopy. Behavior results showed a decrease in ingestive response to NaCl from day 5 to 15 for all groups. Control rats made more ingestive reponses ($p=.058$) than treatment groups. The 6 cGy group showed a significant decrease in ingestive responses to quinine from day 5 to 15. The 12 cGy tongues showed central grooving, thick epithelium, and fewer taste pores. Histologically, radiation effects only the tongue, lower lip and gingiva. Remaining oral tissues, glands and bone remained normal. Typical radiation effects include edema and vascular dilation. New effects seen here are intrafollicular (whisker) hemorrhage, degranulation of tissue mast cells (6 cGy) and depletion of mast cells (12 cGy). The 12 cGy tissues showed more severe acanthosis, atrophic muscle, and abnormal lingual epithelium. In conclusion, behavioral changes can be measured in radiation treated animals. New features of radiation damage to the tongue and oral cavity are described. NIDCD DC00166-02

393 Poster : Histological Profiles in Peripheral Gustatory Systems

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF GABA AND GAD IN FACIAL AND VAGAL NERVE INNERVATED TASTE BUDS OF CHANNEL CATFISH.

Eram M.[1], Michel W.C.[1] *[1]Physiology, University of Utah, Salt Lake City, UT*

We have shown previously established heterogeneity in the metabolic profiles of cells within vagal and facial nerve innervated taste buds, including γ-amino butyric acid (GABA) levels. GABA is a major inhibitory neurotransmitter in the CNS and peripheral neurons but its role in taste buds is unknown. To better understand the role of GABA in peripheral gustatory transmission we examined the distribution of GABA and glutamic acid decarboxylase (GAD)65, the critical enzyme for the biosynthesis of GABA, immunoreactivity (IR) in vagal and facial innervated taste buds using immunohistochemical techniques, electron microscopy and overlay microscopy image analysis. GABA IR was highly variable; most taste bud cells were GABA negative, fewer cells had intermediate or high levels of GABA IR. Vagal innervated taste buds contained more cells with high GABA IR levels than did facial innervated taste buds. GABA IR was also noted in basal cells located below the nerve plexus just above the basa lamina. GAD+ elongated taste cells matched the description of dark taste cells. Overlay microscopy confirmed the presence of GABA in dark taste cells. Basal processes of the GAD+ cells reached the base of the taste bud near the basal cell. GAD IR was not detected in the basal cells. Strong GABA and no GAD IR in basal cells suggest that they may be involved in GABA uptake. The distribution of GABA transporters and receptors is currently being examined to further clarify the role of GABA in gustatory neurotransmission. Supported by NIH DC01418, NS07938 and Willard L. Eccles Charitable Foundation.

394 Poster : Histological Profiles in Peripheral Gustatory Systems

HISTOCHEMICAL EVIDENCE FOR PRESYNAPTIC IONOTROPIC GLUTAMATE RECEPTORS ON PRIMARY GUSTATORY AFFERENT TERMINALS IN GOLDFISH

Huesa G.[1], Finger T.E.[1] *[1]Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO*

Transmission of information from primary afferents can be modulated by neurotransmitter receptors on the primary afferent terminal. Both metabotropic and ionotropic receptors may participate in primary afferent modulation. Ionotropic glutamate receptors involve cation channels whose selectivity varies according to the subunit composition. We rely in the ability of cobalt to pass through Ca++-permeable AMPA/kainate receptors to identify cells possessing such channels. The objective of this work is to test whether primary gustatory afferents possess Ca++ -permeable glutamate receptors that could be involved in the presynaptic modulation of gustatory input. For these experiments, we used goldfish because the primary gustatory nucleus in this species is a large laminated structure in which the layers of primary afferent termination are distinct. Six-30 days after unilateral vagotomy, the fish were anesthetized and the brain was removed for slicing. Vibratome sections (500micrometers) of the living brain were used for the kainate-induced cobalt uptake technique. The vagal lobe of the control side exhibits distinctive layers of punctate Co++ labeling corresponding to the layers of termination of the primary afferents. In contrast, this punctate staining was not present in the lesioned side suggesting that ionotropic glutamate receptors are present on the primary afferent terminal. Thus, these results support the idea that presynaptic ionotropic glutamate receptors may play a role in modulation of primary gustatory afferent transmission. Grant Sponsor: NIH Grant number: DC00147

395 Poster : Pheromones & Social Behavior

THE GENERATION AND CONTROL OF INFORMATION CURRENTS TO EXCHANGE SOCIAL SIGNALS DURING CONSPECIFIC FIGHTS IN CRAYFISH.

Martin A.[1], Bergman D.A.[1], Moore P.A.[1] *[1]Biological Sciences, Bowling Green State University, Bowling Green, Ohio*

Many animals use sensory information to make decisions when regarding social status signals used in subsequent behavioral decisions. Crayfish use chemical signals to determine the past social history and social status of opponents. These signals originate in the urine and are actively released during agonistic encounters. To help facilitate the transfer of information during these encounters, crayfish generate information currents that carry chemical signals to opponents that allow them to sample status signals from an opponent. By using small, neutrally-buoyant particles and light sheets, we have quantified the role of information currents during crayfish fights. Both dominant and subordinant animals generate these currents and use them to send and sample signals. The temporal sequence of information currents correlated with the resulting behaviors, suggests that decisions about social status and subsequent aggressive acts are being performed by detecting pheromones for social status within these information currents.

396 Poster : Pheromones & Social Behavior

SEX, DOMINANCE, AND INDIVIDUAL RECOGNITION SIGNALS IN LOBSTER HIERARCHIES.

Morschauser K.E.[1], Atema J.[1] *[1]Marine Biological Laboratory, Woods Hole, Massachusetts*

Lobsters establish and maintain dominance hierarchies using chemical signals excreted in the urine, including signals for sex, dominance and individual identity. These chemicals allow members of a stable group both to recognize individuals with which they have interacted, and remember the outcome of that interaction. We conducted a unique study on the formation of dominance among a rather large group of animals to examine the effects of the assimilation of past experience on subsequent encounters. Most prior studies only examined dyads. In 13 replicates we placed 6 male and 2 female lobsters, Homarus americanus, naïve to each other, into a 3000L tank. We let them interact according to their own volition, recording all interactions for 4 continuous hours, followed by 10-minute periods each hour for another 4 hours. At the end of 24-hours we recorded for another 10 minutes. In just over one hour major aggression had subsided suggesting that most relationships had been established. Counter to previous results, considerable size differences and physical handicaps did not determine the outcome of an encounter, but prior experience, both immediate and cumulative, did. Thus, dominance significantly results from "confidence" gained from past experiences. In some cases, these effects led to reversals in the hierarchy, influencing the ultimate rank of the animal in the hierarchy. We assume that fight experience, with its copious release of urine signals, allows the animals to link genetically fixed sex and individual identity signals with continuously variable, experience-based dominance signals.

397 Poster : Pheromones & Social Behavior

GENOME-WIDE ANALYSIS OF PHEROMONE-MEDIATED GENE EXPRESSION IN THE HONEY BEE

Grozinger C.M.[1], Robinson G.E.[1] *[1]Entomology and Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL*

Honey bee social organization is predominantly regulated by chemical communication. Using recently developed honey bee cDNA microarrays, we have begun to analyze the molecular mechanisms of pheromonal regulation of honey bee behavior. Here we report on changes in gene expression in the brain due to exposure to queen mandibular pheromone (QMP). QMP is a blend of five identified chemicals that has potent and diverse influences, with effects on brain structure, hormone systems, and several different aspects of behavior including queen rearing and learning. Furthermore, QMP stimulates worker bees to rear brood and delays the transition to foraging behavior. We analyzed changes in brain gene expression caused by QMP exposure, in both lab and field experiments, and identified several sets of co-regulated genes. From this data set it was possible to identify patterns of gene expression which match the differences found in nurses and foragers: genes activated in nurses bees were activated by QMP, while genes activated in foragers were repressed by QMP. Thus, these transcriptional programs may correspond to the initiation of brood-rearing behavior and the repression of foraging behavior. This study will allow us to determine how pheromones affect neural and hormonal pathways that lead to behavioral plasticity. Supported by a Beckman Postdoctoral Fellowship (C.M.G) and grants from the University of Illinois Critical Research Initiatives Program and the Burroughs Wellcome Trust (G.E.R.).

398 Poster : Pheromones & Social Behavior

THE ROLE OF HELIOTHINE HAIRPENCIL COMPOUNDS IN FEMALE *HELIOTHIS VIRESCENS* BEHAVIOR AND MATING

Hillier K.N.[1], Vickers N.J.[1] *[1]University of Utah, Salt Lake City, UT*

Studies on numerous insect species suggest that male-produced sex pheromones may play roles in attracting females; as aphrodisiacs, making females quiescent, or as a means of inhibiting competing males. Male Heliothine moths display abdominal hairpencils during courtship, but the specific effects of the odors released on female behavior have not yet been elucidated. This study investigates the role of male hairpencil compounds in female *Heliothis virescens* mating behavior. Female *H. virescens* were exposed to filter paper loaded with hairpencil extracts of male *H. virescens, Heliothis subflexa*, and *Helicoverpa zea*, and observed for behavioral responses to odors. Single synthetic compounds common to all three species hairpencil blends were also tested. In mating assays between single male and female *H. virescens* it was found that: (i) antennectomized females mated less frequently than normal females, (ii) females mated less frequently with males whose hairpencils had been surgically removed, (iii) females mated with males with ablated hairpencils if a filter paper loaded with 1 male equivalent of *H. virescens* hairpencil extract was presented simultaneously, (iv) this effect was species-specific, as presentation of *H. subflexa* or *H. zea* hairpencil extracts did not restore mating. This study suggests that odors released by male hairpencils are important in mate acceptance by female *H. virescens*, and may play a role in mate choice and species isolation. Studies are currently underway to determine how these odors are detected and processed by the female olfactory system. Supported by USDA NRICGP #1999-03541 & NSF-IBN #9905683 (to NJV).

399 Poster : Pheromones & Social Behavior

PHYSIOLOGICAL AND BEHAVIOURAL STUDIES SUGGEST THE USE OF SEX PHEROMONES DURING REPRODUCTIVE ACTIVITY OF THE ROUND GOBY *NEOGOBIUS MELANOSTOMUS*

Belanger A.J.[1], Petruniak J.[1], Gammon D.[1], Corkum L.D.[1], Li W.[2], Scott A.[3], Zielinski B.[1] [1]*Biological Sciences, University of Windsor, Windsor, Canada;* [2]*Fisheries and Wildlife, Michigan State University, East Lansing, Michigan;* [3]*Center for Environment, Fisheries and Aquatic Sciences, Weymouth, Dorset, United Kingdom*

The round goby is an aggressive and prolific nonindigenous, bottom-dwelling fish that threatens native economically and ecologically important fish in the Great Lakes. Its reproductive success may be mediated by the use of sex pheromones. In this study, we investigated responses from reproductive female round gobies to unknown chemical compounds released by male round gobies. Olfactory physiological responses to water conditioned by male round gobies were assessed through electro-olfactogram (EOG) recordings. The largest EOG responses were from reproductive females to the odor of reproductive males. Reproductive females responded significantly greater than non-reproductive females to water conditioned by reproductive and non-reproductive males. Laboratory behavioural studies showed that females respond by swimming towards odors from conditioned water. Reproductive females were attracted to water conditioned by reproductive males. Overall, these results imply that females ready for spawning activity show increased olfactory mediated sensory responses to and directed movement towards potential mates. Supported by Michigan Great Lakes Protection Fund, NSERC and University of Windsor Faculty of Graduate Studies.

400 Poster : Pheromones & Social Behavior

DISCRIMINATION OF SEMIOCHEMICALS BY THE CHILEAN LIZARD *LIOLAEMUS LEMNISCATUS*: ROLE OF OLFACTION AND VOMEROLFACTION

Labra A.[1], Desfilis E.[2] [1]*Ciencias Ecologicas, Universidad de Chile, Santiago, Chile;* [2]*Universidad de Valencia, Valencia, Spain*

In lizards the vomeronasal organ is proposed as the main receptor involved in discrimination of different semiochemicals (e.g. prey, conspecifics and predators). However, few studies have attempted to determine if olfaction is involved in the chemoreception of those stimuli although many species may have very well developed olfactory bulbs. The role of the olfactory and vomerolfactory systems in the response to own, conspecific and predator semiochemicals, was studied in the lizard Liolaemus lemniscatus. Three groups were established: olfactory peduncle cut (olfactory and vomerolfactory deprivation), olfactory nerve cut (olfactory deprivation) or control (sham; surgery without lesion). Lizards were maintained individually in terrariums, and after a week they were placed individually and randomly in the enclosure of a conspecific, snake (natural predator) or its own. The latency to the first TF, number of TF and total time performing "robotic motion", were recorded. Individuals with the olfactory peduncle cut had longer latency to the first TF, and higher number of TF than the control group. Snake semiochemicals triggered robotic behavior in the 89% of the control lizards and in the 33% of the olfactory nerve cut lizards. Olfactory peduncle cut lizards did not perform this behavior. Both, olfaction and vomerolfaction would be important, at least in the snake chemical discrimination, probable acting in a synergistic way. Funds: IFS 2933-1 (AL).

401 Poster : Pheromones & Social Behavior

CHEMICAL EXPLORATION OF CONSPECIFIC ODORS IN FEMALE OPOSSUMS (*MONODELPHIS DOMESTICA*)

Halpern M.[1], Dombrowski K.[1], Zuri I.[1] [1]*Anat. & Cell Biol., SUNY Downstate Health Sciences Center, Brooklyn, New York*

The gray short-tailed opossum is a South-American marsupial that frequently scent-marks its environment. To date, the significance of opossum body odors in conspecific communication is still unclear.

Investigatory response of 12 female opossums to conspecific body odors (male and female urine, male suprasternal gland, male and female mandibular odor, male and female flank odor) was tested by presenting females simultaneously with two odor stimuli. The time spent in snout contact with the stimuli was analyzed from video recordings.

Except for female and male urine, female opossums investigated all odor sources for significantly longer periods than dH_20 (control odor). Females investigated male suprasternal gland odor significantly longer than most other conspecific odors (male and female urine, male mandible) but not female mandible odor. Odor investigation of male mandible odor was significantly longer than of male or female urine, but did not differ from female mandible odor. Investigation of male urine did not differ from investigation of female urine or female flank odors, but was shorter than of male flank and female mandible odors. Odors of female mandible were investigated more than female urine.

We relate the female interest in male glandular and skin odors to their semi-arid habitat, where glandular marking is advantageous over urine marking as it is less volatile and has minor dehydration effects. A strong response to the odors of female mandibles may indicate the significance of these odors in the context of same-sex communication.

402 Poster : Pheromones & Social Behavior

OLFACTORY MEMORY AND INDIVIDUAL RECOGNITION IN GOLDEN HAMSTERS

Lai W.[1], Yu H.A.[1], Chen A.[1], Johnston R.E.[1] [1]*Psychology Department, Cornell University*, Ithaca, Ny*

The ability to recognize individual conspecifics is crucial in many aspects of an animal's social behavior and in many species olfaction is important for such recognition. Individual recognition requires the association of individually distinct information with memories of past experiences. This type of memory is critical for the stability of relationships and survival. Using golden hamsters, we previously developed a behavioral method for the investigation of individual recognition and used it to study social memory retrieval after aggressive interactions. In this study, we first exposed each male subject with one stimulus donor across a wire mesh. Two hours later, we defeated the subject with the other male donor. We compared the reactions of separate groups of losers to the odors of another male that they had either fought with and lost to or had interacted with across a wire mesh barrier. Several different measures show that losers avoided the arm of the Y-maze holding the winners that beat them. In contrast, losers were attracted to the males that they were merely exposed to. One hour after the behavioral testing, we collected a blood sample and fixed the brain for histology. No significant differences were found in the blood cortisol or testosterone levels between groups. Immunohistochemistry is being carried out to localize c-Fos and Egr-1 expression in the losers' brain and to determinate which brain areas might be involved in social recognition. Our behavioral data suggest that losers can discriminate differences between two equally familiar males and behave differently toward them based on their past experiences. This research was supported by grant number 5R01 MH58001-01A1 from NIMH to R.E. Johnston.

403 Poster : Pheromones & Social Behavior

ACCURATE ODOR DISCRIMINATION IN THE ABSENCE OF ODOR PREFERENCE IN MALE VASOPRESSIN 1B RECEPTOR-KO MICE INDICATES ALTERED SOCIAL MOTIVATION

Kelliher K.R.[1], Zufall F.[1], Wersinger S.R.[2], Young W.S.[2] *[1]Anatomy and Neurobiology, University of Maryland at Baltimore, [2]Section on Neural Gene Expression, National Institute of Mental Health, Bethesda, MD*

Vasopressin 1b receptor knockout (V1bRKO) mice are less aggressive than their wild-type (WT) littermates in several testing paradigms. Here we demonstrate that V1bRKO mice also differ from WT mice in a socially motivated bedding preference test, despite the fact that V1bRKO mice have no difficulty in discriminating between male and female chemosensory cues during an operant testing paradigm. When given a choice between freshly soiled male or female bedding male, WT mice spent significantly more time investigating the soiled female bedding compared to the male bedding. By contrast, male V1bRKO mice spent an equal time investigating both types of bedding. This difference in behavior did not appear to be due to an overall defect in olfactory function since there were no differences between V1bRKO and WT mice in their latencies to detect a cookie buried in clean bedding. To determine whether V1bRKO mice had difficulty in discriminating between male and female chemosensory cues, we used an operant conditioning task using a fully automated liquid dilution olfactometer. This approach showed that male V1bRKO mice were fully capable of discriminating between pooled urine from male mice and pooled urine from female mice. The latencies for V1bRKO mice to learn this task were not different from WT mice, indicating that the two genotypes equally distinguish between male and female odors. We propose that the role of the vasopressin 1b receptor is in the subsequent evaluation of chemosensory cues, rather than their detection.

404 Poster : Pheromones & Social Behavior

MHC-BASED MATE CHOICE IN FEMALE RATS

Shaw-Taylor E., McClintock M.K. *Psychology, University of Chicago, Chicago, IL*

Studies of mate choice and odor preference in mice and humans reveal a role for the major histocompatibility complex, the MHC. Individuals choose mates or prefer the odors of opposite-sex conspecifics who express MHC alleles that are different than their own. MHC-based disassortative mate choice and odor preferences have been shown. Until recently, research on MHC-based preferences focused primarily on differences. There are now studies showing that MHC differences are not always preferred. Two studies have reported female preferences for odors of individuals who express some of the same MHC alleles as themselves, even though odors of more MHC different males were presented. Preferences for the same MHC might suggest an attempt to optimize rather than maximize MHC diversity. An intermediate, optimal amount of MHC diversity has both immune and fitness advantages. Females may choose mates in order to have optimal MHC diversity for offspring. Thus some females may prefer males expressing the same MHC and other females prefer males expressing different MHC. The purpose of this study was to determine if the degree of MHC difference between a female and her potential mates influences her mate choice. Female rats had a choice of mating with PVG congenic males whose MHC varied at one, two or four loci. A partner preference paradigm was used to test mate choice. The hypotheses were that (1) females given a choice between an MHC identical male and an MHC different male would prefer the MHC different male (2) females given a choice between males who were both MHC different would prefer the male with some of the same MHC. Supported by an NIMH MERIT Award to MKM and Hinds Research Fund.

405 Poster : Pheromones & Social Behavior

RESPONSES OF FEMALE MOOSE TO FRACTIONATED URINE OF RUTTING MALE MOOSE (*ALCES ALCES GIGAS*)

Whittle C.L.[1], Bowyer R.[2], Duffy L.K.[3], Preti G.[4], Clausen T.P.[3] *[1]Chemistry & Biochemistry, and Biology & Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska; [2]Biology & Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska; [3]Chemistry & Biochemistry, University of Alaska Fairbanks, Fairbanks, Alaska; [4]Monell Chemical Senses Center, Philadelphia, Pennsylvania*

Olfactory communication and associated scent-marking activities play a major role in the behavioral ecology of many mammals. During the mating season (rut), scent-marking associated with urine of male cervids is an important chemical cue to relay information to conspecifics. Specifically, adult male moose *(Alces alces)* dig rutting pits in which they urinate, and females respond strongly to urine deposited in pits. Results from our previous research demonstrated that when presented with urine from the non-rut and rut periods, female moose preferred the urine from the rut. These results indicate that, during rut, male urine contains constituents that may act as pheromones, which help synchronize and prime estrus. To determine which urinary compounds might function as the male-to-female pheromone, an active pentane extract was separated by preparatory gas chromatography into 3 distinct fractions. The effluents were diluted in pentane and presented to female moose. Bioassays with captive female moose demonstrated that chromatographic fractions containing the compounds unique to the urine of rutting males did not result in significant behavioral responses. We conclude that at least one compound essential for eliciting female response either did not elute or decomposed during the separation. The lack of female response will be discussed. This research was funded in part by NIH grant 1U54NS41069 (NINDS, NIMH, NCRR), NIH grant 5 T32 DC00014-20, NIH/MFP grant 5 T32 MH18882-13, and UNCF/Merck Science Initiative.

406 Poster : Pheromones & Social Behavior

SOCIALLY-RELEVANT CHEMOSENSORY STIMULI SELECTIVELY ACTIVATE POSTERIOR MEDIAL AMYGDALA

Westberry J.[1], Meredith M.[1] *[1]Program in Neuroscience, Florida State University, Tallahassee, FL*

The amygdala receives chemosensory input from olfactory and vomeronasal systems and is involved in social/sexual and emotional responses. Animals distinguish between their own species' social chemosensory signals (reproductive, hierarchical) and signals used by other species for the same purpose. Chemosensory activation in posterior medial amygdala matches this discrimination in male hamsters and our recent evidence implicates intra-amygdaloid inhibition. Both anterior and posterior medial amygdala (MeA/MeP) are activated (Fos, FRA expression) by conspecific, socially relevant, chemosensory stimuli from females (Hamster Vaginal Fluid, HVF, Female Flank Gland Secretion) and from males (Male Flank Gland Secretion). Hamster MeA, but not MeP, is activated by male or female mouse urine, the equivalent signals for mice. MeA without MeP activation also characterizes responses to artificial (electrical or drug) stimulation of the vomeronasal pathway. Since MeP receives much of its input from MeA, lack of excitation from MeA or selective inhibition could prevent response in MeP. We hypothesize that indirect inhibition of MeP results from some patterns of input to MeA. FRA/GABA double-label shows significant activation in GABAergic cells of the medial intercalated nucleus (ICN) when MeP fails to respond, whereas ICN is suppressed during MeP activation. Intrinsic GABA cells of MeA/MeP appear not to be involved. Activation of such cells in MeA does not vary with differences in MeP response and those in MeP are positively not negatively correlated with overall MeP response. Supported by NIDCD grants T32DC00044, DC005813.

407 Poster : Pheromones & Social Behavior

CHEMOSENSORY STIMULATION OF FOS IN MEDIAL AMYGDALA REFLECTS NEURAL ACTIVATION NOT SYNAPTIC ENHANCEMENT.

Blake C.[1], Westberry J.[1], Sirpal S., Weinburg D., Meredith M.[1]
[1]*Program in Neuroscience, Florida State University, Tallahassee, FL*

Immediate-early genes (IEGs) can be activated in neurons by depolarization, probably via calcium influx. Their action as transcription factors could change gene expression to produce increased or decreased sensitivity in the synapses that triggered the activation, or could lead to expression of "housekeeping" genes necessary to return the neuron to its original state. The first of these hypotheses would predict a change in the response of the system to repeated activation over the same input pathway. Repeated stimulation would produce either greater or less IEG expression on the second or third occasion. In the "housekeeping" hypothesis, responses to first, second or third stimulations should be the same. To test these hypotheses for responses in male hamster medial amygdala to chemosensory stimulation, we compared Fos-protein expression following the first or third stimulation with a socially relevant chemosensory stimulus, hamster vaginal fluid. Responses in anterior and posterior medial amygdala to a standard presentation at the same time of day were not significantly different in males experiencing their third stimulation over five days than in males for which this was the first stimulation. Thus, induction of IEGs via the chemosensory pathways does not reflect a modulation of synaptic strength in those pathways. Differences in Fos expression between, for example, sexually naive and experienced animals, could not be interpreted simply as an increase in sensory system sensitivity with stimulus repetition. Supported by NIDCD grants DC005813, T32DC00044, and FSU-CRC.

408 Poster : Pheromones & Social Behavior

OLFACTORY SENSITIVITY FOR ANDROSTENONE IN THREE SPECIES OF NONHUMAN PRIMATES

Laska M.[1], Wieser A.[1], Hernandez Salazar L.[2] [1]*Department of Medical Psychology, University of Munich, Munich, Germany;* [2]*Instituto de Neuro-Etologia, Universidad Veracruzana, Xalapa, Mexico*

Social communication by means of odor signals is widespread among mammals. In pigs, for example, the C19-steroid 5-α-androst-16-en-3-one is secreted by the boar and induces the mating stance in the sow. In humans, the same substance has been shown to be a major compound of body odor and is presumed to affect human behavior. Using a conditioning paradigm, we determined olfactory detection thresholds for androstenone in four pigtail macaques *(Macaca nemestrina),* four squirrel monkeys *(Saimiri sciureus),* and three spider monkeys *(Ateles geoffroyi).* We found that all three species of nonhuman primates are able to detect androstenone at concentrations lower than those reported in pigs and humans. Additional tests, using a habituation-dishabituation paradigm, showed that none of the ten animals tested per species was anosmic to this odorant. These results suggest that androstenone may be involved in olfactory communication in nonhuman primates and that the specific anosmia for this odorant found in ~30% of human subjects may be due to the reduced number of functional olfactory receptor genes compared to nonhuman primates.

409 Poster : Human Olfaction: Pheromones

EFFECTS OF ANDROSTADIENONE ON MOOD AND PHYSIOLOGY INCREASE IN EMOTIONAL CONTEXTS

Bensafi M.[1], Tsutsui T.[1], Sobel N.[2] [1]*Neuroscience, University of California, Berkeley, Berkeley, California;* [2]*Neuroscience, University of California, Berkeley*

Androstadienone (AND) and estratetraenol (EST) are two human sex-steroid derivates. When tested in a non-arousing context, these compounds affected mood and physiology in a sex-specific way. Here we tested whether the effect of these compounds would differ in an arousing context. The effects on mood and physiology of AND, EST and a control compound (baking powder) were assessed in a between-subjects study of 72 subjects (36 f). Baseline measurements were followed by brief compound exposure (6 sniffs) and then by a neutral, sad, happy, or sexually arousing video film. Physiology was monitored constantly and mood was assessed after each film. Memory for film content was assessed after the experiment. During the non-arousing video, AND, but not EST, maintained positive mood in women, but not in men ($F[6,198]=3.51, p<0.003$). No physiological correlates of that mood modulation were observed. During the arousing videos, a double dissociation was observed during the "sad" film: AND, but not EST maintained positive mood (happy) in women ($p=0.05$) and increased negative mood (sad) in men ($p=0.03$). Moreover, AND decreased memory of the "sad" film, but in both sexes ($F[2,66]=3.53, p<0.04$). Effects on physiology were observed during the "erotic" video whereby AND decreased respiration rate specifically in men ($F[2,66]=3.42, p<0.04$). This study has no conclusive findings for EST, but indicates that the sex-specific effects of AND, the most prevalent androstene in human secretions, are more salient in emotional contexts. Funded by the Searle Fellowship.

410 Poster : Human Olfaction: Pheromones

WHAT THE NOSE KNOWS: PREFERENCE FOR HUMAN BODY ODORS AS A FUNCTION OF GENDER AND GENDER PREFERENCE

Martins Y.[1], Preti G.[1], Wysocki C.J.[1] [1]*Monell Chemical Senses Center, Philadelphia, PA*

In preliminary work conducted by Crabtree, Preti and Wysocki (unpublished), it was noted that lesbians responded differently to human body odors obtained from heterosexuals and homosexuals -- lesbians preferred the odor of underarm sweat from other lesbians relative to that from heterosexual females. Based on these results, we hypothesize that there is (a) a detectable difference in body odor based in part upon gender preference and (b) an influence of gender preference on the perception of and preference for the odors of underarm sweat from heterosexual and homosexual individuals. In the present study, heterosexual and homosexual male and female participants indicated their preference for one member of each of the following pairs of underarm sweat odors: (1) heterosexual male vs. homosexual male, (2) heterosexual male vs. heterosexual female, (3) heterosexual female vs. homosexual female, and (4) homosexual female vs. homosexual male. Odor pairs (collected from six members of each group and recombined to form odor classes rather than individuals) were presented 12 times each, counterbalanced across participants, in unlabeled, fragrance-free bottles. Results suggested a difference in the production of odor profile based upon gender preference and, at least for homosexuals, a preference for the odor from other homosexuals. Speculation on the underlying mechanism for differences in odor profiles awaits the results of analytical assessments of collected sweat.

411 Poster : Human Olfaction: Pheromones

ANDOSTRADIENONE'S IMPACT ON EMOTIONAL EXPERIENCE THROUGH MOOD INDUCTION

Freyberg R.[1], Wilson P.[2], Haviland-Jones J.[3] *[1]Rutgers, The State University of New Jersey, Piscataway, New Jersey; [2]International Flavors & Fragrances, Inc., Union Beach, NJ; [3]Rutgers, The State University of New Jersey, Piscataway, NJ*

Previous research has demonstrated the modulating effect of androstadienone (AND) on "naturally" occurring negative mood. An extension of such investigations would examine AND's modulation on deliberately manipulated mood. The current study examines how AND affects participants' emotions during film mood induction, a procedure proven to reliably manipulate target mood. To this end, 160 undergraduate students were exposed to clove oil or fragrance (IFF RD01-014, IFF, Union Beach, NJ) alone or with AND while undergoing happy, fearful, and angry film mood inductions; mood questionnaires were completed before and after viewing each film. Results indicated a significant gender x condition x fear induction procedure interaction, $F(1, 155)=4.79$, $p<0.04$. After the fear mood induction film, men reported similarly higher levels of fear compared to baseline, regardless of odor condition. However, women's fear reports were lower in the experimental AND condition compared to controls. In addition, results suggest that AND caused levels of both positive and negative moods to decrease in women compared to female participants in the control conditions and males in either control or experimental groups, $F(1, 156)=4.26$, $p<0.04$. Such findings extend previous research, which found a decrease in negative mood states. AND's ability to affect induced mood adds support to its growing role as a modulator of emotion. Given our findings, putative pheromones are clearly taking their place among the many factors that both influence and play a part in emotion. This research has been funded by International Flavors and Fragrances, Union Beach, NJ

412 Poster : Human Olfaction: Pheromones

INDIVIDUAL DIFFERENCES IN ODOR SENSITIVITY TO 4,16-ANDROSTADIEN-3-ONE

Lundstrom J.N.[1], Lopes J.[1], Olsson M.J.[1] *[1]Psychology, Uppsala University, Uppsala, Sweden*

Sensitivity to 5alpha-androst-16en-3-one (androstenone) is bimodally distributed in the population. Recently, Lundström et al. (2002) suggested that there may be a bimodal distribution of sensitivity also for the odor of 4,16-androstadien-3-one (androstadienone). This idea was investigated more thoroughly in a test of absolute thresholds. Androstadienone and phenyl ethyl alcohol (PEA) were both diluted in propylene glycol in 16 logarithmic steps. Androstadienone was presented in 180ml squeeze bottles and PEA presented by a sniffin' stick set. Absolute thresholds for androstadienone and PEA were measured for 60 participants (19 to 39 years old) using an ascending staircase method (3-alternative forced-choice). Preliminary data show a bimodal distribution of absolute thresholds for androstadienone and a unimodal for PEA. Moreover, individual thresholds of androstadienone and PEA were uncorrelated. The results suggest that the bimodality found for androstadienone is not based on a bimodality of general olfactory sensitivity in the sample. Support: The Swedish Research Council (HSFR:F0868)

413 Poster : Human Olfaction: Pheromones

INDIVIDUAL DIFFERENCES (IDS) IN THE DETECTION OF HUMAN MOOD FROM AXILLARY ODOR

McGuire T.R.[1], Brahms J.[2], Morris C.[3], Haviland-Jones J.[4] *[1]Genetics, Rutgers, The State University of New Jersey, Piscataway, NJ; [2]Colgate Palmolive, Piscataway, NJ; [3]Biological Sciences, Rutgers, The State University of New Jersey, Piscataway, NJ; [4]Psychology, Rutgers, The State University of New Jersey, New Brunswick, New Jersey*

Previous research demonstrated a low but significant probability that human axillary odors can be correctly labeled with the donors' mood. The current study examines how repeated trials with feedback affect the probability of correctly labeling the mood odors and of establishing reliable IDs. Sixty-one subjects categorized a set of mood odors (*a set is 5 odors: sterile control, 2 happy, 2 fear collected during 24-min video mood inductions on different days from 7 donors; 28 donors total*). There were 15 trials (3 blocks of 5). A "correct" trial required that all 5 odors from the same set be correctly identified. After each trial, feedback was given on correct/incorrect labeling. Correct labeling is above chance for the population ($\chi^2=240.3$, 60 df, $p<.000$). The distribution is bimodal ($g^2= -1.43$, $t= -2.27$, 60 df, $p<.05$). The upper mode is 11/15 trials correctly labeled ($N = 31$) and the lower mode is 0/15 ($N = 30$). Increasing the number of trials and providing feedback provides a larger group of detectors and substantially improves reliable identification of IDs. Apparent anosmia for mood odors cannot be reliably detected with fewer than 6 trials. On trial two reliability of IDs is chance ($r_{tt}= .50$); it increases to $r_{tt}= .75$ by trial 6 and is $r_{tt}= .90$ by trial 15. Use of two or fewer trials substantially underestimates human ability to label mood odors and is unreliable for detecting IDs.

414 Poster : Olfaction & Cognition

OLFACTORY PROCESSING: EFFECTS OF NOSTRIL AND REPETITION

Jonsson F.U.[1], Olsson M.J.[1], Broman D.A.[2] *[1]Department of Psychology, Uppsala University, Uppsala, Sweden; [2]Department of Psychology, Umeå University, Umeå, Sweden*

We investigated lateralization of olfactory processing using monorhinal stimulation in 40 right-handed healthy participants. First, the participants made various judgments of a set of 16 odors. After a short break, the previously presented odors were judged again along with 16 new ones. The dependent measures studied were perceived familiarity, speed of assessing familiarity, valence, and free identification. As opposed to some previous studies, no main differences between nostrils were found for any measure. However, differences between new and previously presented odors could be observed. Previous presentation significantly enhanced the rate of identification, increased the familiarity and the speed of assessing it. This repetition effect on familiarity was more pronounced when tested via the left nostril, which was mainly due to lateralization effects in men. In parallel, in the case of identification, men, but not women, showed significantly higher identification performance as an effect of previous presentation when tested via the left nostril. The present results suggest that in order to understand olfactory lateralization, effects of repetition as well as sex are important factors. (Bank of Sweden Tercentenary Foundation 1998-0270)

415 Poster : Olfaction & Cognition

DO AGE DIFFERENCES IN ODOUR MEMORY DEPEND ON DIFFERENCES IN VERBAL MEMORY?

Moeller P.[1], Wulff C.[1], Koester E.P.[1] [1]*Royal Veterinary and Agricultural University, Frederiksberg, Denmark*

The objective of this study is to investigate the influence of verbal mediation on odour memory. Incidental and intentional memory for unknown odours were tested in 39 young (Age M = 25.6, SD = 4.3; 21 female, 18 male) and 41 elderly (Age M = 66.7, SD = 6.1; 29 female, 12 male) subjects who also rated the 40 odors on pleasantness, familiarity and intensity and finally tried to provide a name for the odours in a separate presentation. Thus it was verified whether they had developed a name for the odours. For the incidental memory test they received 10 out of the 20 odors which they had rated the previous day interspersed with 10 new odors and were asked to indicate which of these odors they had smelled before. The same procedure was followed for the intentional learning procedure with 20 new odors, but this time they were explicitly instructed to try to remember them. Our data show that incidental odour memory is at least as good in the elderly as in the young, but that the young do much better on the intentional test, whereas the elderly do not improve. There is no correlation between memory of an odour and the ability to name it. In conclusion, we have demonstrated the existence of a human sensory odour memory system, which functions independently of verbal information. Identification is not a necessary prerequisite for the functioning of this memory system. Furthermore, this system does not deteriorate with age. This work was carried out with financial support from the European Commission Quality of Life Framework Programme. QLK-1999-00010.

416 Poster : Olfaction & Cognition

NEGATIVE AFFECTIVITY ENHANCES REACTIVITY DURING REPEATED ODORANT EXPOSURE

Maute C.[1], Dalton P.[1] [1]*Monell Chemical Senses Center, Philadelphia, Pennsylvania*

Dispositional factors, such as negative affectivity (NA), can influence responding to odorous compounds by directly altering the individual's processing goals and memory retrieval. NA may also indirectly influence autonomic responses, because emotional and perceptual effects from previous chemical exposures can increase the autonomic arousal elicited by re-exposure to the odor stimulus. In this study we investigated the interaction of NA with repeated exposures to an odorous chemical. Subjects scoring high on NA were compared with subjects scoring low before, during and after two 30-minute exposures to isopropyl alcohol. Before and after each exposure session, subjects rated sensory and health symptoms; nasal volume was assessed using , acoustic rhinometry. During each exposure, subjects rated a variety of different attributes of their experience, including the intensity, irritancy and quality of the odor as well as perceived stress, anxiety and annoyance. Objective measures of ocular hyperemia were obtained for a subset of the subjects for comparison with self-reports of eye irritation. Consistent with predictions, subjects who scored high on NA rated more symptoms after each exposure and also showed less adaptation on the second exposure than subjects who scored low on NA. The results suggest that the influence of personality factors on sensory and somatic interpretations may become reinforced with repeated experience. Supported by NIH DC 03704

417 Poster : Olfaction & Cognition

WHEN SENSES AND COGNITIONS COLLIDE: EFFECTS OF AMBIENT ODOR ADMINISTRATION ON EVALUATIONS OF WRITING SAMPLES

Smith J.[1], Raudenbush B.[1] [1]*Psychology, Wheeling Jesuit University, Wheeling, West Virginia*

Particularly with the advent of essay questions on standardized tests, the influence of factors other than writing quality on evaluation has become more important. Prior research on essay writing shows complex interactions between gender, sex, penmanship, and expectations that significantly affect scores; other studies demonstrate a correlation between attractiveness and test scores for females. Even if the reader was blind to the aforementioned variables and possessed no prior information about the writer, other factors could negatively or positively impact an evaluation, such as mood or the presence of an unpleasant odor (e.g. the lingering smell of cigarette smoke). Based on previous research demonstrating odors have significant effects on the human nervous system, even in the absence of awareness to these odors, the present study examines whether the presence of an odor affects perceived quality of a written piece and the personal enjoyment of reading it. One hundred eighty-nine participants were exposed to peppermint, lemon, dimethyl sulfide, or a non-odored control condition while reading an essay in either typed, poorly handwritten, or clearly handwritten form. Participants then completed questionnaires related to its quality (e.g. introduction, presentation, overall) and their mood. Dimethyl sulfide odorant lead participants to rate their experience as less enjoyable, although the perception of quality was not significantly affected.

418 Poster : Olfaction & Cognition

FRAGRANCE EXPECTANCIES AND PERCEIVED EFFECTS: A STUDY OF SUBJECTIVE AND OBJECTIVE RESPONSES

Koenitzer J.C.[1], Naqvi F.[1], Dalton P.[1] [1]*Monell Chemical Senses Center, Philadelphia, PA*

Cognitive schemas can be as influential on the perceptual response to an odorous volatile as the physical properties of the sensory stimulus itself. However, interactive effects of these two factors are largely unknown. This study examined the relationship between fragrance expectancies and subsequent prolonged exposure to a novel odor. Sixty participants were assigned to one of three bias groups (n = 20/group) in which the odorant 4-acetyl carene (carenko) was characterized as either a (1) natural fragrance or (2) standard test odorant or (3) synthetic chemical. All participants were exposed to carenko on two visits for 15 min. in an environmental chamber. During the one-week interim between visits, half of the participants were exposed to carenko in their homes. Subjective endpoints included ratings of odor quality and health symptoms, both of which were measured during exposure visits and every other day between visits. For exposure visits, objective endpoints included respiration rate and cortisol levels (a hormone considered a reliable marker of stress). In addition, odor detection thresholds (ODTs) were obtained pre- and post- exposure on both visits for carenko and a control odorant. As anticipated, the results indicate that subjective ratings of odor quality and health symptoms are susceptible to bias. Surprisingly, when coupled with prolonged exposure, bias seemed also to effect sensitivity: for those receiving in-home exposure, an interaction was found such that ODTs increased for the natural group but decreased for the synthetic group from visit one to visit two. Supported by the AirCare Division of CSPA and NIH RO1 DC 03704

419 Poster : Olfaction & Cognition

AUTOBIOGRAPHICAL MEMORY: INFLUENCES OF AGE AND CUE TYPE

Willander J.M.[1], Kuldkepp E.[1], Larsson M.[1] *[1]Department of psychology, Stockholm University, Stockholm, Sweden*

The purpose of this study was to further our understanding regarding odor-cued autobiographical memories (AM). Of particular interest was to determine whether odor evoked memories are older, more emotional and more vivid (i.e., the Proustian hypothesis) than are memories evoked by other sensory stimuli. Also, we wanted to explore whether age influenced AM. Young (M = 25.5; range 21-36 yrs) and old (M = 73.1; range 69-78 yrs) adults were presented with verbal and olfactory cues and asked to relate an isolated event in the past history associated with the specific cue. Preliminary results show that olfactory evoked memories were older (Mean age at event = 11.7 years, SD=6.5) than word-cued memories (Mean age at event = 16.5, SD=7.4) in younger subjects. In contrast, the age of memories did not differ reliably for the odor-evoked (M=24.4, SD=15.2) and verbally evoked (M=29.2, SD=23.5) AM among the older adults. Regarding qualitative aspects in AM, participants ratings' of emotional quality indicated no main effects of age or cue type. Of interest to note, however was the significant age by cue type interaction reflecting that older adults experienced odor-cued memories as more emotional relative to younger subjects, whereas no age difference was observed in emotional arousal for memories evoked by verbal cues. Likewise, odor-cued memories elicited stronger feelings of being brought back in time among the older adults as compared with the younger participants, whereas no age effect was obtained for the word-cued memories. Finally, older adults rated overall the vividness of evoked AM higher than younger adults for both cue types. Support: Swedish Research Council (F0647/2001)

420 Poster : Olfaction & Cognition

SYNTHETIC ODORS AND THE STROOP EFFECT

Buschmann-Maiworm R.[1], Winkel S.[2], Schurian W.[2] *[1]Dep. of Psyhcology, University, Münster, Germany; [2]University of Münster, Münster, Germany*

The aim is to investigate the effect of natural ,congruent' odors on the so called Stroop effect. The question is if a congruent or incongruent odor (yellow or green smelling odor) enhances or delays the recognition of color word written in its own color. In the 1st pre study we asked people what smells blue, red green and yellow and what food smells blue, red, green yellow. About 70 % said yellow means lemon, about 60 % said green means green apple. Considering literature we chose citral for lemon and 1-hexanal for green apple. In our 2nd pilot study we tested the reverse association for citral with lemon and hexanal with green apple. The Stroop test in the main experiment was delivered by the ERST computer. We have three treatment groups: 1% citral (3,7-Dimethylocta-2,6-dien-1-al; cis/trans, Aldrich), 10 % citral, 0.5 % 1-hexanal(Sigma) and 1 control group (prop.gly.). One odor or the odourless control was sprayed on a sheet of paper. It lay on the table beside the subject during the whole session. Each subject was tested once under one expr. condition. Very interesting is the congruent exp. condition: word yellow written in yellow plus odor 'yellow' in comparison to 'no odor; and also the olfactory incongruent condition of yellow written in yellow smelling 'green'. We started analysing the data. Tendencies show a shorter recognition time in the word color congruent setting with congruent odor (yellow written in yellow with citral). The effects could be stronger if we could use odors which are much stronger associated wit a single color.

421 Poster : Olfaction & Cognition

EDIBILITY PRIMING: TOWARDS A PROCEDURE FOR THE MEASUREMENT OF REPETITION PRIMING EFFECTS IN OLFACTION

Olsson M.J.[1], Willander J.M.[2], Jonsson F.U.[1] *[1]Department of Psychology, Uppsala University, Uppsala, Sweden; [2]Department of Psychology, Stockholm University, Stockholm, Sweden*

Repetition priming procedures are often used to investigate implicit (non-conscious) memory. In olfactory cognition, no standard procedure for repetition priming has been developed and validated. This presentation depicts a series of experiments investigating the primability of edibility judgments. The results indicate that (1) edibility judgments are facilitated when repeated. The facilitation concerns latency rather than correctness of the judgments. (2) This latency facilitation occured regardless of whether the odor tested could be identified or not. (3) Using the edibility priming procedure, unilateral presentation of odors can serve to tap hemispheric differences. (4) As for visual priming, edibility priming is long lasting. (5) Edibility priming is dissociated from explicit memory in terms of forgetting curves. (6) Edibility judgments of odor words do not produce shorter latencies for later judgments of the corresponding odors. This result rules out certain types of conceptual processing. Altogether, the results suggest that the assessment of latencies of edibility judgments for repeated and new odors yields a robust and valid procedure for the investigation of implicit memory in olfactory cognition. (Bank of Sweden Tercentenary Foundation 1998-0270)

422 Poster : Olfaction & Cognition

EARLY LEARNING ABOUT THE SENSORY PROPERTIES OF TOBACCO

Mennella J.A.[1], Forestell C.A.[1], Henry C.[1], Beauchamp G.K.[1] *[1]Monell Chemical Senses Center, Philadelphia, PA*

Previous research in our laboratory revealed that early experience with odors influence later emotional and hedonic responses to the odor. The present study focused on 3- to 9-year-old children to determine whether the hedonic response and the latency to make a hedonic judgment to the odor of tobacco smoke was related to the smoking habits of their parents. Age appropriate, game-like tasks that were fun for children and minimized the impact of language development were used to examine their response to a variety of odors, one of which was that of tobacco smoke. Preliminary analyses revealed that the child's response to the odor of tobacco varied as a function of maternal smoking and age of child. These findings suggest that some early learning about tobacco is based on sensory experiences and anchors it to children's experiences at home. This research was supported by Pennsylvania Research Formula Fund.

423 Poster : Olfaction & Cognition

DIRECTED ATTENTION INCREASES SENSITIVITY TO TARGET BUT NOT BACKGROUND ODORS

Dalton P.[1], Diamond J.[1], Breslin P.A.[1] [1]*Monell Chemical Senses Center, Philadelphia, Pennsylvania*

Repeated test exposures to peri-threshold odors can lead to dramatic sensitivity increases among young women, but not men. Despite daily exposure to hundreds of peri-threshold odors, females generally do not exhibit greater sensitivity on initial threshold tests suggesting that factors other than mere exposure control the sensitivity changes following repeated olfactory detection thresholds (ODTs). To test whether focused attention is sufficient to induce sensitization, we compared the influence of directed attention on a `target' odor relative to an `irrelevant' odor cue by obtaining repeated ODTs against a background odor. ODTs for dichorhinic mixtures of benzaldehyde (B) and citralva (C) were obtained from 10 females on 10 test sessions. The concentration of the `target' odor (B or C) was varied in a step-wise fashion, while the `irrelevant' background odor (C or B) was held at a concentration just above its ODT. Subjects sniffed from two pairs of bottles during each trial - one pair contained the dichorhinic mixture, the other only background odor - and selected the pair containing the target odor. Thresholds for each odorant alone were obtained at the first and last two sessions. ODTs for the `target' odor decreased by an average of 3.5 log steps across the ten sessions and continued to decrease in the final two sessions. Sensitivity to the `irrelevant' background odor during the last two sessions did not significantly change from the first two tests. These results suggest that attention is an important moderating influence on exposure-induced sensitivity and that mere exposure to peri-threshold stimuli in young women is not sufficient to induce large-scale changes in odor sensitivity. NIH RO1 DC 03704 & DC 02995

424 Poster : Olfaction & Cognition

ODOR-EMOTIONAL CONDITIONING: EFFECTS ON BEHAVIOR

Herz R.S.[1], Schankler C.[1], Beland S.[1] [1]*Psychology, Brown University, Providence, Rhode Island*

This study tested the hypothesis that odors can become conditioned to emotions and subsequently influence performance. Two experiments were conducted with female college students. In experiment 1, participants were exposed to a novel ambient odor while they played a frustrating computer game. After a 20 min break, participants were taken to another room and given three cognitive word puzzles, each containing a number of problems. The puzzle tasks were performed under three ambient odor conditions: (1) the same odor as the computer game; (2) a different novel odor from the computer game; (3) no odor. Results revealed that participants who performed the puzzle tests in the same odor condition, spent significantly less time working on the problems than participants in the different odor or no odor groups. Experiment 2 confirmed that the effects for the same odor participants were due to lower persistence, as opposed to greater efficiency, as time spent working on problems that ended up not being solved was significantly less than in the other groups. Experiment 2 also ruled out the possibility that the effects were due to longer exposure to the `same' odor, as a condition in which participants were initially exposed to an ambient odor while watching a neutral video and then performed the puzzle tests with the same odor showed similar performance to the different and no odor groups. These findings extend previous data in children and demonstrate a general phenomenon; that emotions can become conditioned to odors and subsequently exert a directional influence on behavior.

425 Poster : Clinical Issues in the Chemical Senses

DIFFERENTIAL STRESS-RELATED GENE EXPRESSION IN OLFACTORY BULBS OF ELDERLY AND AD SUBJECTS

Getchell M.L.[1], Vaishnav R.A.[1], Buch S.K.[1], Li H.[2], Shah D.S.[3], Getchell T.[4] [1]*Anatomy and Neurobiology, University of Kentucky College of Medicine, Lexington, KY;* [2]*Statistics, University of Kentucky, Lexington, KY;* [3]*Family Practice, St. Luke Hospital, University of Wisconsin Medical School, Milwaukee, WI;* [4]*Physiology, University of Kentucky College of Medicine, Lexington, KY*

Alzheimer's disease (AD) is associated with oxidative stress. To assess the stress pathways involved in the olfactory bulb (OB) in AD, we used SuperArray Stress and Toxicity macroarrays. OBs were removed with short postmortem delays from 3 elderly non-demented control subjects and 3 confirmed AD patients, all females. Macroarrays were hybridized with cDNA probes synthesized from OB total RNA. Of the 96 genes represented, 27 were expressed at detectable levels. After normalization to ribosomal protein L13a, 17 genes exhibited expression levels at least 30% higher in OBs from ADs than controls. These genes were associated with heat shock, oxidative stress, DNA damage, proliferation, and senescence. None of the detectable genes were expressed at lower levels in ADs than in controls. The gene expressed at the highest level in all subjects was the inflammatory regulator macrophage migration inhibitory factor (MIF), which was upregulated by ~25% in AD subjects. We confirmed protein expression and localization for several upregulated genes by Western blotting and immunohistochemistry. These results demonstrate that gene expression associated with specific stress pathways is higher in OBs of AD patients than in age-matched controls. Supported by NIH NIA R01 AG16345 (MLG) and NIH P50-AG05144 (AD Research Center, Sanders-Brown Center on Aging).

426 Poster : Clinical Issues in the Chemical Senses

A DESCRIPTION OF AXONAL ABNORMALITIES OBSERVED IN HUMAN OLFACTORY EPITHELIUM.

Holbrook E.H.[1], Leopold D.A.[1], Schwob J.E.[2] [1]*Otolaryngology-Head and Neck Surgery, University of Nebraska Medical Center, Omaha, NE;* [2]*Anatomy & Cellular Biology, Tufts University, Boston, MA*

Analysis of human olfactory epithelium obtained from subjects with normal as well as abnormal olfactory function has been described. Characterization of this tissue has mostly focused on the cellular architecture superficial to the basal lamina. Given the patchy topography of olfactory epithelium in the human, observed changes in olfactory axons and the fila olfactoria may be more informative of the status of the epithelium as a whole. A description of the olfactory axons in patients with olfactory disorders of various etiologies may then provide valuable information about the mechanisms of olfactory disease in humans and help to establish a diagnosis in certain individuals. Methods: A retrospective review was performed on olfactory epithelium biopsies obtained from patients with and without various olfactory disorders. Analysis of olfactory tissue included immunohistochemistry and/or electron microscopy specifically focused on olfactory axons and the fila olfactoria. Results from olfactory testing were correlated with histologic findings when testing results were available. Results/Conclusions: Various changes in olfactory axons are noted in patients with olfactory disorders. A group of patients appear to have normal caliber fila olfactoria, however the olfactory axons are replaced with collagen. In other groups, neuromas are abundant but display differences in location within the epithelium. A shift to a higher proportion of immature olfactory axons within fasicles is also observed in a number of specimens. A summary of histologic findings, olfactory ability, and type of olfactory disorders is provided in order to detect trends.

427 Poster : Clinical Issues in the Chemical Senses

COMPREHENSIVE ASSESSMENT OF OLFACTORY FUNCTIONS IN YOUNG MALE SCHIZOPHRENIA PATIENTS

Rupp C.I.[1], Klimbacher M.[1], Lechner T.[1], Walch T.[1], Kemmler G.[1], Scholtz A.[2], Hinterhuber H.[1] *[1]Department of Psychiatry, University Clinics of Innsbruck, Innsbruck, Austria; [2]Department of Otorhinolaryngology, University Clinics of Innsbruck, Innsbruck, Austria*

The clinical relevance of olfactory impairment in schizophrenia has been described in terms of a potential vulnerability marker, a tool for assisting localisation of cerebral pathology, as well as a tool to classify patients into meaningful discrete subtypes. Past years' research consistently reported impaired odor identification. However, impaired identification has been found in a variety of neuropsychiatric diseases. Its neural substrate remains unclear. Our goal was to assess unirhinally different olfactory tasks. We hypothesized that dysfunctions in olfactory processing in schizophrenia clearly go beyond identification deficits. We compared performance of basic olfactory functions (detection threshold, quality discrimination), odor identification, and judgements about everyday odors (familiarity, edibility, pleasantness) between young male patients and healthy controls. Aside from impaired identification, schizophrenia patients showed deficits in basic olfactory functions, as well as in specific types of odor judgements. Thus, dysfunctions included necessary components, and tasks, suggested to represent steps in the identification process. The findings of left and right nostril disturbances suggest dysfunctions in neural circuitry of olfaction within both hemispheres. Comprehensive assessment of various olfactory functions along several key lines, including clinical syndromes, as well as pathognomonic specificity, may provide valuable information with regard to the clinical relevance described in the assessment of olfactory functioning in schizophrenia. Jubiläumsfondsprojekt no. 6576 / OENB

428 Poster : Clinical Issues in the Chemical Senses

HAZARDOUS EVENTS ASSOCIATED WITH IMPAIRED OLFACTORY FUNCTION

Santos D.V.[1], Reiter E.R.[2], Dinardo L.J.[2], Costanzo R.M.[1] *[1]Physiology, Virginia Commonwealth University, Richmond, Virginia; [2]Otolaryngology-Head and Neck Surgery, Virginia Commonwealth University, Richmond, Virginia*

Patients with olfactory loss are at increased risk of experiencing hazardous events; notably, kitchen related accidents, inability to detect a fire or gas leak, and ingestion of toxic substances. However, there is sparse quantitative information on the number of patients who actually experience these adverse events. Olfactory function testing and hazard interviews were conducted for 445 patients evaluated at a university medical center smell and taste clinic over a period of 18 years (1983-2001). Of these patients 32.4% experienced at least one of the four olfactory-related hazardous events. Incidents that occurred while cooking represented 45% of the total number of hazardous events reported. Additional hazards assessed included: inability to detect a gas leak (23%), ingestion of spoiled food (23%), and inability to smell a fire (9%). Of the 76% of patients found to have some degree of olfactory impairment on testing, 30% were anosmic. For the anosmic group, 45.2 % experienced at least 1 of the 4 hazardous events, while only 19.0 % of the normosmic group reported the same. The decreasing frequency of hazardous events in the severely hyposmic (34.1%), moderately hyposmic (32.8%), and mildly hyposmic (24.2%) groups suggest that there is a correlation between degree of impairment and frequency of hazardous events. Supported by NIH DC00165

429 Poster : Clinical Issues in the Chemical Senses

SUITABILITY OF THE ODOR STICK IDENTIFICATION TEST FOR JAPANESE IN PATIENTS SUFFERING FROM OLFACTORY DISTURBANCE

Kobayashi M.[1], Nishida K.[2], Oishi M.[2], Majima Y.[1], Maeda T.[3], Furuta S.[3], Nakamura S.[3], Saito S.[4], Takashima Y.[5] *[1]Otorhinolaryngology,, Mie University School of Medicine, Tsu, Mie, Japan; [2]Otorhinolaryngology, Mie University School of Medicine, Tsu, Mie, Japan; [3]Otorhinolaryngology, Maeda Hospital, Matsusaka, Mie, Japan; [4]NeuroScience Research, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan; [5]Olfaction, Takasago International Corporation, Tokyo, Tokyo, Japan*

We studied suitability of the Odor Stick Identification Test for the Japanese (OSIT-J) in patients suffering from the olfactory disturbance. In 120 patients with olfactory disturbance, ranging in age from 12 to 85 years, there were statistically significant correlations among the odor identification rate on the OSIT-J, the Japanese standardized olfactory test (T&T olfactometry) and the grade of subjective symptom scores. In every patient treated for the olfactory disturbance, the OSIT-J reflected the grade of recovery from olfactory disturbance as T&T olfactometry. The identification rate on the OSIT-J also correlated with results of the intravenous Alinamin(R) test significantly. As for the percentages of the correct recognition of the odors, the menthol and curry odors were recognized in high rate and the orange and wood odors were in low rate in the patients. While the OSIT-J includes 13 kinds of odors, the number of odors may be reduced to a minimum of 5 kinds of odors since the odor-reduced OSIT-J also correlated with T&T olfactometry and the subjective symptom scores as well as the 13-odor OSIT-J. We conclude that the OSIT-J is suitable not only for a screening of olfactory disturbance but also for a practical use in clinical otorhinolaryngology.

430 Poster : Clinical Issues in the Chemical Senses

MIDDLE TURBINATE FENESTRATION: A NEW ENDOSCOPIC SINUS SURGERY APPROACH FOR THE IMPROVEMENT OF OLFACTORY FUNCTION

Miwa T., Tsukatani T., Uramoto N., Yata T., Furukawa M., Horikawa I.[1] *[1]Otorhinolaryngology, Kanazawa University, Kanazawa, Japan*

Olfactory disturbance is a major symptom associated with chronic sinusitis, and chronic sinusitis is one of the most frequent causes of olfactory disorders. The majority of patients with olfactory disorders caused by chronic sinusitis improve with treatment, including the use of endoscopic sinus surgery. Although in many cases olfactory improvement is achieved when the olfactory cleft is widened by lateral deviation of the middle turbinate, in some cases relapse of the olfaction disturbance and sinusitis occurs due to narrowing of the middle meatus in the long term observation. We developed a new endonasal sinus surgery technique for the olfactory disturbed patient and report its procedure and long-term results. The most important step in this procedure is to open the basal lamella of middle turbinate just anterior of the superior turbinate and to make a new pathway for airflow to the olfactory mucosa. Measurements of olfactory acuity in this procedure were the same as the former technique, and there were no deterioration of the olfactory function in the long-term follow up study. In conclusion, the middle turbinate fenestration technique provides a new treatment option for those patients with olfactory disturbance.

431 Poster : Clinical Issues in the Chemical Senses

LOCAL AND SYSTEMIC APPLICATION OF CORTICOSTEROIDS IN THE TREATMENT OF OLFACTORY LOSS

Heilmann S.[1], Hummel T.[1] [1]*Smell and Taste Clinic, Dept. of ORL, University of Dresden Medical School, Dresden, Germany*

This unblinded study aimed to evaluate olfactory function in patients receiving steroids either locally (group A) or systemically (group B). Investigations before and after treatment included clinical examinations, standardized history, and measures of olfactory function (butanol odor threshold, odor discrimination, odor identification). We included patients with olfactory loss after viral infections, patients with apparent sinunasal disease, and "idiopathic" patients with no obvious cause of dyosmia. In group A, mometasone spray [MO] was prescribed for 1 to 3 months to 92 patients; only 37 patients (21 f, 16 m; mean age 58 yrs) returned for re-investigation after treatment (after 21 to 330 days). Although odor identification tended to increase ($p=0.05$), MO did not significantly improve olfactory function. In group B, oral prednisolone was prescribed to 84 patients in decreasing doses over 21 days (starting dose: 40 mg); 55 patients returned for re-investigation (25 f, 30 m, mean age 57 yrs). Improvement of dysosmia was seen over all diagnostic categories ($p<0.001$). Dysosmia also improved in patients with postviral olfactory loss ($p=0.05$) and in those with idiopathic dysosmia ($p=0.008$). Summarily, MO seems to have little effects on dysosmia, especially when considering long-term changes. As the poor response to MO is partly due to the spray´s disposition pattern in the nasal cavity, improvent of the administration technique is needed. However, the discrepancy between the responses to local and systemic steroids may also point towards a central-nervous site of action.

432 Poster : Clinical Issues in the Chemical Senses

TASTE/SMELL DYSFUNCTION IN CARDIAC SURGICAL PATIENTS

Stahl B.[1], Bartoshuk L.M.[2], Funk M.[3] [1]*Yale University, New Haven, CT;* [2]*Surgery, Yale University, New Haven, Connecticut;* [3]*School of Nursing, Yale University, New Haven, CT*

Cardiac surgical procedures are the most frequently performed operations in the United States. Commonly occurring complications include cardiac dysrhythmias, wound infections, pleural effusions, and neurological deficits. However, in a preliminary study the most common complaint (90% of patients) was a problem with taste. Taste problems can delay recovery by decreasing dietary intake at a time when optimal nutrition is needed for wound healing. The primary aim of this study is to examine the nature and incidence of taste/smell dysfunction in patients who have coronary artery bypass graft (CABG), and/or valve replacement/repair surgery and thoracic aneurysm repair. Patients rated taste intensities with the general Labeled Magnitude Scale preoperatively, immediately postoperatively, and 2 weeks post-discharge. Ratings of NaCl were significantly reduced at the immediate postoperative testing. There were trends toward reduced ratings at the immediate postoperative period for sucrose and quinine. Retronasal olfaction (assessed with jelly beans) and orthonasal olfaction (assessed with ivory soap, coffee, peanut butter) were significantly decreased over the testing times. The preliminary results conclude that there are statistically significant changes in taste, salty predominantly, as well as changes in orthnasal and retronasal olfaction after cardiac surgery. These findings hamper postoperative recovery in patients after cardiac surgery by creating a condition in which foods become tasteless and difficult to eat. Also because the foods are flavorless, patients are more likely to add additional condiments (for example salt and sugar), which may cause increases in serum sodium and glucose levels, further hampering complete recovery.

433 Poster : Clinical Issues in the Chemical Senses

TASTE STRIPS: VALIDATION AND CLINICAL APPLICATION OF A NEW PROCEDURE FOR THE QUANTITATIVE ASSESSMENT OF GUSTATORY FUNCTION

Mueller C.[1], Kobal G.[2] [1]*Otorhinolaryngology, University of Vienna, Vienna, Austria;* [2]*Pharmacology, University of Erlangen-Nuremberg, Erlangen, Gemany*

Assessment of gustatory sensitivity in a clinical setting is the prerequisite for correct diagnosis and adequate treatment of taste dysfunction. Despite of this no taste test has been established for the routine clinical testing. The aim of the present study was to create and validate a new procedure which is easy to administer. This procedure is based on strips made from filter paper which were impregnated with different taste solutions. The following concentrations were used for the taste strips: Sweet: 40/ 20/ 10/ 5% sucrose. Sour: 30/ 16.5/ 9.1/ 5% citric acid. Salty: 25/ 10/ 4/ 1.6% sodium chloride. Bitter: 0.6/ 0.24/ 0.096/ 0.038% quinine-hydrochloride. These strips are placed on the tongue and subjects are asked to identify the taste quality. The investigation involved 69 subjects (36 female, 33 male, mean age 29 years, range 15-75 years). Each subject received eighteen taste strips (four concentrations of each taste quality plus two blanks) in a pseudo-randomized sequence starting with the lowest concentrations. Results from this new procedure correlated significantly with the results of the well established three-drop-technique ($r69=0.67$). Repeated measures indicated good reproducibility of the results for the taste strips ($r69=0.68$). These data suggest the usefulness of this new technique in routine clinical practice. Major advantages are long shelf-life, convenience of administration, short time needed for testing and the possibility to test each side of the tongue separately. Additional clinical data will be presented including test results of patients suffering from xerostomia.

434 Poster : Clinical Issues in the Chemical Senses

DYSGEUSIA: MORE PREVALENT THAN SUSPECTED?

Bartoshuk L.M.[1], Duffy V.B.[2], Kveton J.F.[1], Snyder D.J.[1] [1]*Surgery, Yale University School of Medicine, New Haven, CT;* [2]*Dietetics, University of Connecticut, Storrs, CT*

Dysgeusia (chronic taste) can reflect the presence of an unsuspected tastant (e.g., medication in saliva). Alternatively, the taste may originate in the nervous system (i.e., taste phantoms). A neural structure mediating taste may be abnormally stimulated (e.g., pressure from a tumor), or, because various loci in the taste system inhibit one another, damage in one area can lead to release of inhibition producing abnormal activation at another. Severe dysgeusia motivates a patient to seek health care. We suggest that dysgeusia also occurs in milder forms. Attendees (N=5395) at lectures completed questionnaires that included the item: "Do you ever have persistent salty, sweet, sour, or bitter tastes in your mouth?" Each attendee also rated the bitterness of PROP paper (filter paper impregnated with 1.6 mg 6-n-propylthiouracil) using the general Labeled Magnitude Scale (valid for measuring differences across subjects or groups). Percentage responses to the question were: never=57.2%, occasionally=29.8%, sometimes=12.3% and always=0.7%. Those reporting dysgeusia differed significantly in a number of ways from those who did not: Mild dysgeusics were younger, included more women, perceived more bitterness from PROP, and had lower body mass indices than controls. The questionnaires also asked about head trauma and otitis media, since these are associated with taste damage; mild dysgeusics showed significantly more severe histories of both. A separate group of mild dysgeusics (N=286) given a spatial taste test to assess potential gustatory nerve damage showed some localized losses. This suggests that some forms of mild dysgeusia may be mediated by localized damage to taste. (NIH/DC00283)

435 Poster : Clinical Issues in the Chemical Senses

COLON CANCER AND GENETIC VARIATION IN TASTE

Basson M.D.[1], Bartoshuk L.M.[2], Dichello S.Z.[3], Weiffenbach J.[4], Duffy V.B.[5] *[1]Surgery, Wayne State University School of Medicine, Detroit, MI; [2]Surgery, Yale University, New Haven, Connecticut; [3]Nutrition, Veteran's Administration Hospital, Wallingford, CT; [4]NIDCR, National Institutes of Health, Bethesda, MD, U.S.; [5]Dietetics Program, University of Connecticut, Storrs, Connecticut*

Colon cancer risk is increased by low vegetable intake and high body mass index (BMI), a measure of adiposity. Interestingly, vegetable intake and BMI have been associated with PROP (6-n-propylthiouracil) bitterness, a measure of taste genetics. Here we explore associations between PROP and colon cancer and whether they are mediated through diet. Men who were screened for colon cancer (N=250) with colon/ sigmoidoscopies rated PROP bitterness (1.6 mg delivered via filter paper) on the general Labeled Magnitude Scale (gLMS). This scale (derived from the LMS, Green et al, 1993) is a line with adjectives placed such that it has ratio properties with the top "strongest imaginable sensation of any kind." PROP bitterness correlated significantly with polyp number ($r = 0.22$, $p<.01$) for men above 65 years; no correlation ($r = 0.01$) existed for those under 65 years. BMI was significantly greater for the men over 65 with polyps (chi sq= 6.969, df=2, $p<.05$). The PROP, BMI and polyp associations were also present in those without taste-related pathology. A subset (N=87) were interviewed with a food frequency instrument. In answer to "About how many servings of vegetables do you eat per day or per week, not counting salad or potatoes," those with zero or one polyp reported greater intake than those with two or more polyps (chi sq= 4.407, df=1, $p<.05$). PROP status may increase colon cancer risk by influencing diet. (VA MAVERIC and NIDCD funded)

436 Poster : Clinical Issues in the Chemical Senses

IPSILATERAL TASTE INTENSITY PERCEPTION DEFICITS IN A LEFT INSULAR STROKE PATIENT

Simmons K.B.[1], Mak Y.E.[1], Gitelman D.R.[1], Small D.[1] *[1]Cognitive Neurology and Alzheimer's Disease Center, Northwestern University, Chicago, IL*

Previous research has implicated the insula in gustatory processing, including taste intensity perception, and has suggested that taste information projects ipsilaterally from the tongue to the this region of the cortex. We determined whole mouth taste detection and recognition thresholds and left and right tongue suprathreshold taste intensity perception in a 70-year old right-handed male with a discrete left posterior superior insular stroke. As predicted, his taste intensity ratings were significantly lower on the left (ipsilateral) compared to the right (contralateral) tongue ($p=0.003$). Intensity perception and identification detection and recognition thresholds of citric acid were normal in whole mouth stimulation. On a control task, his ratings of visual shade intensity were normal, demonstrating that the perceptual impairments were not generalized. These results are in accordance with previous evidence that ipsilateral projections to the left insula mediate taste intensity perception on the left tongue.

437 Poster : Clinical Issues in the Chemical Senses

USE OF TASTANTS TO FACILITATE WEIGHT LOSS

Gallant-Shean M., Hirsch A.R. *Smell & Taste Treatment and Research Foundation, Chicago, I*

OBJECTIVE: Obesity is of epidemic proportion; more than one third of the American population is overweight. Despite this, effective treatment remains elusive. Chemosensory techniques for weight loss have been explored: Hirsch found orthonasal olfactory stimuli induced a 2.1% reduction in body mass per month over a six-month period. METHODS: To assess if retronasal olfaction and taste may also affect weight, 108 overweight subjects (13 to 348 pounds, average 197 pounds, body mass index (BMI) of 31) with an average age of 48 (range 1 to 64) sprinkled two different tastants per month (cheddar cheese/cocoa, onion/spearmint, horseradish/banana, ranch/strawberry, taco/raspberry, parmesan cheese/malt) for six consecutive months. Weight and hedonics were obtained on a monthly basis. This was compared to a control group of 100 traditional diet program enrollees (average weight 192 pounds, BMI 30). RESULTS: 92 (85.1%) completed the study, losing an average of 5.6 pounds (range: -3 to +6) (2.8% decrease in BMI) whereas the control group gained an average of 1.1 pound. The average improvement in the experimental group compared to the control group was -6.7 pounds. CONCLUSION: Flavors may act both through taste and the retronasal olfactory route, affecting chemosensation, and thus, facilitate weight loss.

Wednesday April 9, 2003

10:00am-12:00pm
Educational Outreach
GWIZ science center

12:00-3:30
Executive Committee
Executive Board Room

5:00-7:30
Registration
Prefunction Area

6:30-8:00
Opening Buffet
Salons A, B

Welcome & Awards

Givaudan Lecture
Bert Hoelldobler
Multicomponent Signals in Ant Societies
Salons C, D, E, F

Social Gathering, & Cash Bar
Prefunction Area

Organization meeting for students with travel awards
Salon G

Thursday April 10, 2003

Morning Coffee 7:30-9:00

8:00-9:45
Slide Session
Animal Behavior & Olfaction
Salons C, D, E, F

8:00-12:00
Poster session
- Olfactory Neurogenesis & Cell Death
- Development of Gustatory Systems
- Gustatory Processing
- Sweet Taste
- Bitter Taste
- Trigeminal Chemoreception & Irritation
- Sensory Evaluation & Consumer Research

Salons A, B, G, H

10:00-12:15
Symposium
In Sync: Temporal Coding and Encoding Time in the Olfactory System
Salons C, D, E, F

12:30-2:00
Minority and Clinical Travel Awardee Luncheon
Executive Board Room

3:30-5:00 NIH Workshop
Funding Opportunities for New Investigators
Salon C, D, E, F

7:00-8:15
Slide Session
Human Psychophysics Taste & Trigeminal
Salons C, D, E, & F

7:00-11:00
Poster session
- Chemical Ecology
- Olfactory CNS Processing
- Retronasal Olfaction
- Perception of Mixtures & Multimodal Stimuli
- Aging

Salons A, B, G, H

8:30-10:30
Symposium
Hanging by a Thread: Scaffolds in Signal Transduction
Salons C, D, E, F

Friday April 11, 2003

Morning Coffee 7:30-9:00

8:00-10:00
Slide Session
Functional Organization of Gustatory Systems
Salons C, D, E, F

8:00-12:00
Poster session
- Animal Behavior: Tracking & Orientation
- Olfactory Transduction
- Olfactory Coding in the Periphery
- Neurotransmitters in Olfactory CNS
- Neural Plasticity & Regeneration

Salons A, B, G, H

10:15-12:15
Symposium
Interplay of Olfaction & Emotion Systems
Salons C, D, E, F

12:15-2:00
Business Meeting
Salons C, D, E, F

2:00-4:00
Softball Game
Fruitville and Lockwood Ridge Road

6:00-8:00
AChemS 25th Anniversary Symposium: "Perspectives on the Chemical Senses"
Salons C, D, E, F

8:00-8:30
Buses leave hotel for Ringling Museum

8:30-11:00
AChemS 25th Anniversary Reception
at the Ringling Museum

Saturday April 12, 2003

Morning Coffee 7:30-9:00

8:00-10:00
Slide Session
Olfactory Function in Health & Disease
Salons C, D, E, F

8:00-12:00
Poster session
- Olfactory Receptor Genes
- Olfactory CNS Coding
- Gustatory Transduction
- Development of Olfactory Systems
- Salt & Sour Taste
- Umami Taste

Salons A, B, G, H

10:15-12:20
Symposium
Patterning in Olfactory Systems: How Much is Pre-Specified?
Salons C, D, E, F

12:15-2:00
Clinical Luncheon
The Keys

2:00-4:00
Workshop on Olfactometry
Salons C, D, E, F

7:00-8:00
Slide Session
Molecular Basis of Sweet Taste
Salons C,D,E,F

7:00-11:00
Poster session
- Olfaction in Animal Behavior
- Animal Behavior: Taste & Feeding
- Oscillations & Synchronization in Olfaction
- Olfactometry & Gustometry
- Vomeronasal Chemoreception
- Human Olfaction: Psychophysics

Salons A, B, G, H

8:15-10:30
Presidential Symposium: Biology & Chemistry of Floral Scent
Salons C,D,E,F

Sunday April 13, 2003

Morning Coffee 7:30-9:00

8:00-9:15
Slide Session
Development of Chemoreceptor Cells
Salons C,D,E,F

8:00-12:00
Poster session
- Olfactory Rec Cells
- Histological Profiles in Peripheral Gustatory Systems
- Pheromones & Social Behavior
- Human Olfaction: Pheromones
- Olfaction & Cognition
- Clinical Issues

Salons A, B, G, H

9:30-11:45
Slide Session
Functional Organization of Olfactory Systems
Salons C,D,E,F

NOTES

Morning Slide Sessions *and* **Morning Symposia**
8:00am – 12:15 noon
Salons C, D, E, F

Morning Poster Sessions & Exhibits
8:00 am – 12:00 noon
Salons A, B, H, G

Evening Slide Sessions *and* **Evening Symposia**
7:00 – 10:30pm
Salons C, D, E, F

Evening Poster Sessions & Exhibits
7:00 -11:00 pm
Salons A, B, G, H

Morning Coffee
7:30-9:00 Prefunction Area

Mid-Morning Coffee
9:30-10:00 am
Prefunction Area

Evening Break
8:00-8:30 pm
Prefunction Area

Cash Lunch Cart
after morning sessions
Prefunction Area

April 21-25, 2004
April 13-17, 2005