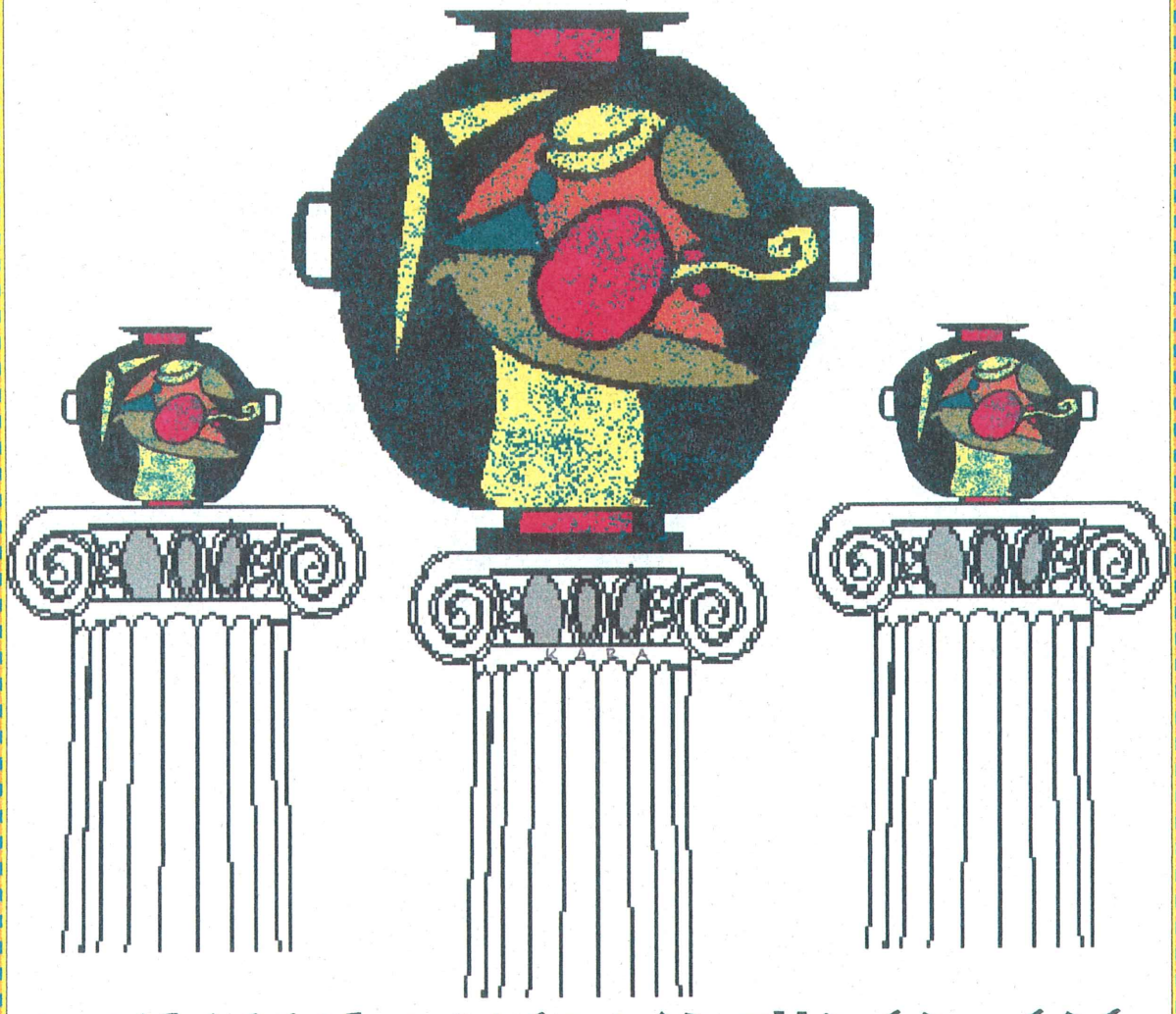
THE ASSOCIATION FOR CHEMORECEPTION SCIENCES
PRESENTS
IMPRESSIONS ON A NEW CENTURY
OF OLFACTION AND TASTE



A COLOSSAL FEAST FOR THE SENSES

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Lectures and Symposia

THE DROSOPHILA ODORANT-BINDING PROTEIN LUSH IS REQUIRED FOR NORMAL OLFACTORY BEHAVIOR

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Insects like Drosophila segregate olfactory neurons into discrete hair-like sensilla. Each sensillum contains one to four olfactory neurons that project dendrites into the hollow, fluid-filled core of the hair. In different sensilla, different repertoires of odorant-binding proteins (OBPs) are secreted into the lymph bathing the dendrites. Odorant binding proteins are, therefore, potentially important regulators of chemical specificity for olfactory neurons in insects. We have identified LUSH, a member of the Drosophila OBP family, and have generated and characterized mutants defective for expression of this OBP. LUSH represents the only known OBP mutant in any species. We demonstrated that LUSH mutants have defective olfactory behavioral responses to a small subset of odorants. The behavioral defects are completely reversed by introducing a LUSH transgene into the mutants. These results demonstrate a clear role for an OBP in chemical discrimination. We are generating transgenic flies that misexpress various OBP members in the Drosophila olfactory sensilla to identify any olfactory behavior effects. These experiments will be discussed.

CANDIDATE ODORANT RECEPTORS IN DROSOPHILA AND THEIR CELLULAR EXPRESSION

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The DOR genes of Drosophila are likely to encode a large family of odorant receptors. The genes are predicted to encode seven-transmembrane-domain proteins, and different family members are expressed in different subsets of ORNs. As one test of the possibility that DOR proteins are in fact odorant receptors, we have raised antibodies against the product of the DOR22A.2 gene, a gene whose RNA is restricted to a small subset of neurons in the dorso-medial region of the third antennal segment. The antibody labels a small subset of sensilla in this dorso-medial region. The staining co-localizes with dendrites, as expected for an odorant receptor. We have found that different DOR genes initiate expression at different times in olfactory system development. Some genes are first detected late in antennal development, as found for the OBP gene OS-E. By contrast, other DOR genes are expressed much earlier, at a time when the antennal nerve is increasing in diameter. These results are consistent with the possibility that a subset of DOR genes plays a role in axon guidance. An intriguing problem in olfaction concerns the regulation of odorant receptor genes. How do individual neurons select, from among an enormous repertoire of receptor genes, which genes to express? We have found evidence that the Acj6 POU domain transcription factor plays a role in this process. In acj6 mutants, a subset of DOR genes is not expressed normally, and a subset of ORNs undergoes alterations in odor-specificity. We have found that several other POU genes are also expressed in the olfactory system, suggesting the hypothesis that receptor gene choice may be governed in part by a combinatorial code of POU domain transcription factors.

THE MOLECULAR LOGIC OF OLFACTION IN DROSOPHILA

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Drosophila fruit flies display robust olfactory-driven behaviors with an olfactory system far simpler than that of vertebrates. Endowed with approximately 1500 olfactory receptor neurons, these insects are able to recognize and discriminate among a large number of distinct odorants. Candidate odorant receptor molecules responsible for this specificity were identified by complimentary approaches of differential cloning and genome analysis (1, 2). The Drosophila odorant receptor (DOR) genes encode a novel family of proteins with seven predicted membrane-spanning domains, unrelated to vertebrate or nematode chemosensory receptors. There are on the order of 50 or more members of this gene family in the Drosophila genome, far fewer than the hundreds to thousands of receptors found in vertebrates or nematodes. DOR genes are selectively expressed in small subsets of olfactory neurons, in expression domains that are spatially conserved between individuals, bilaterally symmetric, and not sexually dimorphic. Double in situ RNA hybridization with a number of pairwise combinations of DOR genes fails to reveal any overlap in gene expression, suggesting that each olfactory neuron expresses one or a small number of receptor genes and is therefore functionally distinct. How is activation of such a subpopulation of olfactory receptor neurons in the periphery sensed by the brain? In the mouse, all neurons expressing a given receptor project with precision to 2 of 1800 olfactory bulb glomeruli, creating a spatial map of odor quality in the brain (3). We have employed DOR promoter transgenes that recapitulate expression of endogenous receptors to visualize the projections of individual populations of receptor neurons to subsets of the 43 glomeruli in the Drosophila antennal lobe (4). The results suggest functional conservation in the logic of olfactory discrimination from insects to mammals.

1. P.J. Clyne, *et al.*, Neuron 22, 327-38 (1999).
2. L.B. Vosshall, H. Amrein, P.S. Morozov, A. Rzhetsky, R. Axel, Cell 96, 725-36 (1999).
3. P. Mombaerts, *et al.*, Cell 87, 675-86 (1996).
4. P. P. Laissue, *et al.*, J Comp Neurol 405, 543-52 (1999).

FUNCTIONAL GENOMICS OF ODOR-GUIDED BEHAVIOR IN *DROSOPHILA MELANOGASTER*

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We use *Drosophila melanogaster* as a model system to investigate how the coordinated expression of ensembles of genes regulates odor-guided behavior. *P*-element insertional mutagenesis in an isogenic strain of flies combined with a statistical assay that enables reliable quantification of olfactory avoidance behavior resulted in the identification of a set of 14 smell-impaired (smi) loci, 12 of which were suitable for further characterization. Quantitative genetic analysis of double heterozygotes constructed from parents homozygous for different smi genes revealed extensive epistatic interactions among this group of smi loci. *P*-element insertional mutagenesis tags the *smi* loci for cloning enabling expression levels of gene products to be quantitatively correlated with the behavioral phenotype. Initial characterization of three *smi* loci, *smi35A*, *smi60E* and *smi97B*, revealed new proteins that are essential for the coordination of olfactory signal processing, including a novel kinase (*Dyrk2*), a voltage-gated sodium channel of previously unknown function, and a novel, yet uncharacterized, leucine-rich-repeat protein likely to play a role in postsynaptic signaling.

GENETIC DISSECTION OF FOOD SEARCH BEHAVIORS IN DROSOPHILA

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Two questions underlie the research in our laboratory. 1) How do genes and their proteins regulate normal individual differences in behavior? and, 2) How do genes and their proteins act within the organism in response to the environment to regulate changes in an individual's behavior? We address these questions using Drosophila food search behavior as a model. The talk will focus on two genes, *foraging (for)* and *scribbler (sbb)* each of which that have distinct behavioral functions in food search behavior. Foraging has two naturally occurring *rover (forR)* and *sitter (fors)* alleles that confer differences in food search behavior. Larvae carrying the rover allele exhibit long foraging trails in a large yeast patch and tend to move between depleted food patches while homozygous sitter larvae locate the closest food patch and remain feeding on it. Similarly adult rover flies walk significantly farther from a recently consumed sucrose drop than sitter flies whose higher turning rate promotes revisiting and keeps the fly near the drop. The *foraging* gene encodes a cGMP dependent protein kinase (PKG) and rovers have higher PKG activities than do sitters. Neuronal activity also differs in these natural variants. Unlike normal larvae, *scribbler* mutant larvae exhibit increased turning in the absence of food and relatively straight movements on food. Our recent cloning of *scribbler* shows that it is a large gene encompassing >50 kb of genomic DNA *sbb* RNA is found in the embryonic and larval nervous systems and the larval imaginal discs. We restored normal larval behavior in a *scribbler* mutant background by targeting expression of a normal *scribbler* transgene to presynaptic neurons. *Scribbler* encodes a novel nuclear protein.

ANATOMY AND PHYSIOLOGY OF PIRIFORM CORTEX SUGGEST FUNCTIONAL ROLES EQUIVALENT TO HIGHER ORDER CORTEX IN OTHER SENSORY SYSTEMS

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Despite its traditional designation as primary olfactory cortex, the piriform cortex has few parallels with other primary sensory areas. New data from analysis of projections from individual intracellularly injected cells, populations of anterogradely and retrogradely labeled cells, immunocytochemical markers, recording of single unit responses to odor, and cellular-level visualization of odor-evoked activity with Fos antiserum, indicate that the piriform cortex consists of four or more subdivisions. Comparisons to the visual system reveal parallels between piriform cortex and both secondary (extrastriate) and sensory association areas. The olfactory bulb appears to subserve functions like those carried out by primary visual cortex.

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DYNAMIC ODOR RECEPTIVE FIELDS IN RAT PIRIFORM CORTEX

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An explosion of work in the past decade has begun to clarify the nature of odor coding at the receptor sheet and the olfactory bulb levels. Current models of odor coding at these peripheral stages involve multiple receptors responsive to specific molecular moeities which project in precise spatial patterns to olfactory bulb glomerular sheet. Mitral/tufted cells, using both spatial and temporal information, display odor/molecular receptive fields which produce odor specific activity patterns projected to the primary olfactory (piriform) cortex.

Recent work in the anterior piriform cortex (aPCX) has demonstrated that cortical odor receptive fields are highly dynamic, showing rapid changes of both firing rate and temporal patterning within relatively few inhalations of an odor, despite relatively maintained, patterned afferent input. For example, repeated or prolonged, unreinforced odor presentation results in a rapid reduction (habituation) of odor-evoked firing rate, odor-evoked post-synaptic potential amplitude and respiration-entrained firing patterns in aPCX neurons. The change in odor-evoked activity is correlated with an odor-specific depression of afferent (lateral olfactory tract) synaptic efficacy. Both odor-evoked responses and afferent synaptic depression recover within 2 min following termination of odor stimulation.

Importantly, odor habituation of aPCX responses is odor specific, with minimal cross habituation to either similar molecular compounds, markedly different compounds or between binary odor mixtures and their components. This is in dramatic contrast to results with mitral/tufted cells, which show strong cross habituation to similar molecular compounds. The results of cross-habituation studies suggest that mitral/tufted cells have broad receptive fields (respond to multiple, though perhaps chemically similar odors) due to a loose coding of receptor activity, while aPCX neurons have broad receptive fields due to convergence of relatively independent lines, each of which can be modified by experience.

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NEUROMODULATION AND THE FUNCTIONAL DYNAMICS OF PIRIFORM CORTEX

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Performance in odor memory tasks is impaired by blockade of acetylcholine and norepinephrine receptors. My research has focused on linking behavioral effects to the blockade of specific modulatory influences at a cellular level. Brain slice experiments demonstrate effects of acetylcholine and norepinephrine on excitatory synaptic transmission (Hasselmo and Bower, 1993; Hasselmo et al., 1997; Linster et al., 1999), inhibitory synaptic transmission (Patil and Hasselmo, 1999), neuronal adaptation (Barkai and Hasselmo, 1994) and long-term potentiation in the piriform cortex (Patil et al., 1998). Computational modeling demonstrates how modulatory effects in the piriform cortex and olfactory bulb might enhance the encoding of odor information (Linster and Hasselmo, 1997; Hasselmo et al., 1997), and behavioral predictions of these models have been supported by experimental work (Linster and Hasselmo, 1999; DeRosa and Hasselmo, in press). New projects are focusing on the role of rhythmic activity observed in the olfactory system for odor encoding and consolidation.

RULES OF FORMATION OF THE OLFACTORY REPRESENTATIONS FOUND IN THE ORBITOFRONTAL CORTEX OLFACTORY AREAS IN PRIMATES

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In primates the secondary and tertiary olfactory cortices are in the orbitofrontal cortex. Neuronal recordings show that the five prototypical tastes sweet, salt, bitter, sour, and umami are represented here; that the pleasantness or reward value of taste and odour is represented as shown by satiety experiments; that a representation of the flavour of food is formed; and that this is built for at least 35% of neurons by learned association or odour with taste. Oral somatosensory inputs also provide for a representation of fat in the mouth, and olfactory inputs can activate some of these neurons.

In investigations of whether there are similar areas in humans, fMRI results show a gustatory representation in the medial orbitofrontal cortex distinct from the olfactory representation in the right orbitofrontal cortex, and a separate representation of affectively positive somatosensory stimuli in a different region of the human orbitofrontal cortex (Francis et al, 1999). It is shown that olfactory sensory-specific satiety is represented in the human orbitofrontal cortex (O'Doherty et al, 2000), and this is evidence that the pleasantness of odors is represented in the human orbitofrontal cortex. The primate orbitofrontal cortex is thus involved in taste and olfactory processing, in the control of food intake, and also in emotion and emotion-related learning.

THE ROLE OF NERVES IN THE INDUCTION OF TASTE BUDS: A CONCEPT REVISITED

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Although recent experimental evidence from both amphibians and mammals indicates that taste buds develop from local epithelia, the role of nerves in this process remains controversial. Taste buds of salamanders differentiate in the complete absence of nerves, while taste bud development in rodents is clearly affected by disruption of an intact nerve supply. How can we reconcile these data to come to a clearer understanding of the general mechanisms involved in the genesis of vertebrate taste buds? One way to approach this problem is to ask what is meant by the phrase "induction of taste buds". Among developmental biologists the term induction implies that one set of cells emits a signal that changes the fate of cells receiving the signal. Induction in the context of developing taste buds has been assessed typically by examining the distribution of mature taste buds after experimental manipulation of a hypothetical inducer, usually the nerve supply. However, the genesis of taste buds during development must include a number of stages, including 1) the formation of some type of taste bud progenitor cell(s) from among otherwise indifferent epithelial cells, 2) the production of immature taste receptor cells through asymmetric division of these progenitor cells, and 3) cytodifferentiation of taste receptor cells. Each of these hypothesized events in the building of a taste bud may require one or more inductive signals from one or more inductive tissues. In this talk, I will explore the data pertaining to taste cell lineage and turnover, as well as consider mechanisms by which other sensory organs arise during development. As a result of this discussion, I hope to generate testable hypotheses of taste bud development which ultimately will allow a general understanding of taste bud formation and the role of nerves in this process.

Supported by NIDCD DC03947.

EARLY DEVELOPMENT AND DIFFERENTIATION OF TASTE ORGANS AND INNERVATING GANGLIA: INDEPENDENT AND INTERDEPENDENT REGULATORY FACTORS

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The lingual gustatory organs (papillae and taste buds) and sensory ganglia that innervate the tongue arise separately in the mammalian embryo and begin to differentiate without papilla - ganglion interactions. As ganglion cell bodies extend neurites that grow toward and eventually reach the tongue, there is opportunity for exchange of signals that can reciprocally regulate subsequent differentiation and development. To identify factors that influence development of both the gustatory organs and their innervating sensory ganglia, we use *in vitro* culture systems of rat embryo tongue and ganglion tissue. In organ cultures of the embryonic tongue that exclude intact sensory innervation, the fungiform and circumvallate papillae form and differentiate in appropriate locations and patterns. Other regulatory factors for papilla development include diffusible protein products of *sonic hedgehog, bone morphogenic protein, distal-less,* and *neurotrophin* genes. When teratogenic, steroidal alkaloids that disrupt sonic hedgehog signal transduction are added to tongue cultures, papillae develop in increased numbers and atypical locations, suggesting that inter-papilla tongue epithelium is released from inhibitory regulation. Expression of neurotrophins in specific compartments of the developing tongue and gustatory papillae indicates a role for these molecules as major target factors that may influence not only survival, but also morphological and functional differentiation of innervating ganglion cells. Exposure of geniculate, trigeminal and petrosal ganglion explants to exogenous neurotrophins in culture demonstrates embryonic, stage - dependent influences on neurite extension and morphology of the growing tip. Furthermore, when ganglia are cultured with a neurotrophin that is most effective at promoting neurite outgrowth, compared with a less effective neurotrophin, specific effects on ganglion cell neurophysiology are observed. In summary, whereas gustatory papillae initially form without direct influence of sensory ganglion cells, and ganglia begin to differentiate without target contact, subsequent early molecular interactions have potential for directing the nature and extent of gustatory organ and ganglion cell development.

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TROPHIC FACTORS IN THE DEVELOPING PERIPHERAL GUSTATORY SENSE ORGANS

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Brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) mRNAs are expressed in developing and adult rodent tongue and have been shown to be important for the proper development of the lingual gustatory and somatosensory innervation, and taste bud development in rodents. Distinct, specific, and, in some instances overlapping patterns of BDNF and NT-3 mRNA expression are found in the developing and adult human tongue, gustatory papillae, and taste buds. Neurotrophin 4 (NT-4), another member of the neurotrophin family of neurotrophic factors, plays an important role for the survival of geniculate neurons. These factors have also been shown to elicit neurite outgrowth from cultured cranial ganglia, and BDNF seems to be synaptogenic for BDNF-responsive gustatory fibers. Other growth factors, such as epithelial growth factor (EGF), have been proposed to be important factors for the development of taste buds. Much work has been done in order to understand and characterize the molecules and mechanisms involved in the development of sensory organs for the sense of taste, and there is much work to be done. As it has been agreed upon for almost hundred years, taste buds develop from the lingual epithelium, they are found in predefined and prespecialized areas, and they require interaction with predominantly gustatory fibers for development in mammals, however, not in amphibians. Different types of organ culture and transplantation approaches can be utilized to study the interaction of the naïve gustatory epithelium and the ingrowing gustatory fibers, some of which I will touch upon in this symposium. In addition, molecular biology techniques, specifically transgenic approaches, will also provide us with strong tools for understanding these interactions in more detail.

NEUROTROPHIN RECEPTORS IN SINGLE GENICULATE GANGLION NEURONS

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Recent studies (Nosrat & Olson, 1995) have shown that embryonic rat gustatory epithelium, the target for sensory nerves originating in the geniculate ganglion, contained the mRNA of the neurotrophin, Brain Derived Neurotrophic Factor (BDNF). The Nosrat and the Oakley labs have shown that BDNF knockout mice exhibited severe deficiencies in taste bud development. Others have shown the geniculate ganglion in BDNF null mutant mice had a 50% reduction in the number of geniculate ganglion neurons. Double knockout mice lacking BDNF and NT-4 exhibit a 90% loss of geniculate ganglion neurons and have malformed and poorly innervated taste bud structures, implicating both neurotrophins and their common receptor, trkB, as being critically important in the survival of the neurons and development of taste buds. The mRNA of another neurotrophin, NT-3, was found in the non-gustatory epithelium surrounding taste buds but not in the taste buds themselves. Mutant mice lacking the gene for NT-3 showed a 47% loss of geniculate ganglion neurons, suggesting that this neurotrophin and its receptor, trkC, also play a role in geniculate ganglion neuron survival. In a recent study (Cho & Farbman, 1999) we showed that whole rat geniculate ganglia from 3 week old animals contained the mRNAs for trkB, trkC, and small amounts of trkA. We now present data that single neurons, dissected and isolated from stained sections of 3 week old rat geniculate ganglia, contain mRNAs for either trkB, or trk C, but not both. The observation that significant number of geniculate ganglion neurons express trkC and no other trks suggests that NT-3 is an important trophic factor for survival of this sub-population of neurons. Moreover, the observation that these neurons contain no demonstrable trkB suggests that they are not trophically dependent on BDNF in taste buds.

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NEURON/TARGET MATCHING BETWEEN CHORDA TYMPANI NEURONS AND TASTE BUDS DURING POSTNATAL RAT DEVELOPMENT

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During postnatal rat development, a relationship is established between the size of individual taste buds and number of innervating neurons. This relationship between taste bud size and number of innervating neurons is not apparent until P40, when taste bud size reaches maturity. The focus of this presentation will be to demonstrate that the number of neurons innervating taste buds at P10, when taste bud size is small and relatively homogeneous, predicts the size that the respective taste bud will become at maturity. Moreover, while there is some neural rearrangement of taste bud innervation from P10 to P40, rearrangement does not impact on the relationship between taste bud size and innervating neurons. That is, the neurons that maintain contact with taste buds from P10 through P40 accurately predict the mature taste bud size. Therefore, the size of the mature taste bud is determined by P10 and relates to the number of sensory neurons that innervate it at that age and the number of neurons that maintain contact with it throughout the first 40 days of postnatal development. A working model will be presented to explore the underlying cellular mechanisms.

This work is supported by NIH grant DC 00407.

NOVEL MEANS OF RECEPTOR FUNCTION: RAMPS, HETERODIMERISATION AND TRANSCRIPTION FACTORS

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We and others have recently shown that a number of 7-transmembrane G protein-coupled receptors require additional accessory proteins to ensure their correct folding, cell surface localisation and ability to couple to intracellular signalling networks. Initially, in an attempt to expression clone the calcitonin gene-related peptide (CGRP) receptor, we identified a family of three single transmembrane spanning proteins which we termed 'receptor activity modifying proteins' (RAMPs) which enabled the calcitonin receptor-like receptor (CRLR) to be transported to the cell surface and to function as a CGRP or adrenomedullin receptor. We also found that following recombinant expression, the recently cloned $GABA_B$-R1 receptor, which was reported to mediate metabotropic actions of the inhibitory neurotransmitter GABA, was localised to intracellular membranes, was expressed as an immature glycoprotein and could not convey responses to GABA. Motif searches of the $GABA_B$ sequence revealed the presence of a recognised protein-protein interaction promoting coiled-coil domain within the C-tail. Hence, yeast two-hybrid analysis was performed using the C-terminal tail of $GABA_B$-R1 as bait against a brain cDNA library in order to search for an accessory protein. The screen identified a close homologue of $GABA_B$-R1, termed R2. We found that co-expression of $GABA_B$-R2 with R1 generated a mature, cell surface localised, high affinity $GABA_B$ receptor, hence suggesting that the functional $GABA_B$ receptor is made up of a heterodimer consisting of two related 7-transmembrane proteins, $GABA_B$-R1 and $GABA_B$-R2. In addition, the same yeast two-hybrid screen identified two related transcription factors, CREB2 and ATFx . In recombinant systems and in neurones we found that agonist activation of the $GABA_B$ receptor led to a translocation and accumulation of CREB2 from the cytoplasmic processes into the cell nucleus, resulting in activation of gene transcription. This mechanism of direct modulation of gene transcription is a unique observation for G-protein coupled receptors and may play a role in long term changes in the nervous system.

MOLECULAR MECHANISMS OF 7TM RECEPTOR ACTIVATION

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Abstract Not Available

PROBING THE STRUCTURAL BASES OF PHARMACOLOGICAL SPECIFICITY IN THE DOPAMINE D2-LIKE RECEPTORS

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Conserved features of the sequences of dopamine receptors and of homologous G-protein-coupled receptors point to regions, and amino acid residues within these regions, that contribute to their ligand binding sites. Differences in binding specificities among the catecholamine receptors, however, must stem from their nonconserved residues. Using the substituted-cysteine accessibility method (SCAM), we have identified the residues that form the surface of the water-accessible binding-site crevice in the dopamine D2 receptor. Of ~80 membrane-spanning residues that differ between the D2 and D4 receptors, only 20 were found to be accessible, and 6 of these 20 are conservative aliphatic substitutions. In a D2 receptor background, we mutated to the aligned residues in the D4 receptor, individually or in combinations, the 14 accessible, non-conserved residues. We also made the reciprocal mutations in a D4 receptor background. The combined substitution of 4-6 of these residues was sufficient to switch the affinity of the receptors for several chemically distinct D4-selective antagonists by three-orders of magnitude in both directions (D2 to D4-like and D4 to D2-like). The mutated residues are in the second, third and seventh membrane spanning segments (TM2, TM3, TM7) and form a cluster in the binding-site crevice. Mutation of a single residue in this cluster in the second membrane-spanning segment was sufficient to increase the affinity for clozapine to D4-like levels. We can rationalize the data in terms of a set of chemical moieties in the ligands interacting with a divergent aromatic microdomain in M2-M3-M7 of the D2 and D4 receptors.

A COMPUTATIONAL GENOMICS ROADMAP OF THE HUMAN OLFACTORY SUBGENOME

Lancet D.[1], Glusman G.[1], Avidan N.[1], Ben-Asher E.[1], Gilad Y.[1], Horn-Saban S.[1], Khen M.[1], Olender Z.[1], Segre D.[1], Fuchs T.[1] [1]*Weizmann Institute of Science, Rehovot, Israel*

Olfactory receptors (ORs) constitute the largest multigene family in multicellular organisms. Their evolutionary proliferation has been driven by the need to provide recognition capacity for millions of potential odorants with arbitrary chemical configurations. The complete extent of this family is not known for any specie. Due to the progress of Human Genome Project, Homo sapiens will likely be the first vertebrate species in which the complete OR repertoire will be known. Yet, to date, no systematic account has been produced for the ever-increasing arsenal of human OR genes. We present here an analysis of the 224 human OR proteins, the largest compendium known today for any specie, which sheds light on their structure, function and evolution. More than half of this collection are newly detected sequences stemming from cloning experiments or data mining. A nomenclature system approved by the world Human Genome Organization (accompanied paper) allowed the analysis of the philogenetic relationships in this superfamily. The computational package presented will allow facile dissemination and public availability of the entire olfactory receptor subgenome when the first draft of the human genome is completed next year. It will also be highly instrumental for genomic analyses of ORs in other species and of other multigene families. This effort is currently being merged with projects aimed at generating DNA microarrays that contain OR sequences and polymorphisms, that will allow a comprehensive elucidation of inter-human variability in OR sequences. GeneCards, a compendium of human genes with automatic data mining, developed at our Genome Center, will be modified to accommodate the very large number of human OR genes. Together, the above methodologies will provide a comprehensive computational genomics tool kit for studying the OR gene repertoire.

Posters and Slides

2 Slide

EFFECTS OF ALKALINE PH ON THE APPARENT MOLAR COMPRESSIBILITIES OF SWEETENERS

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Magnesium hydroxide solution (saturated at 20°C) was added to aqueous solutions of sucrose or sodium saccharin in order to measure solution parameters in relation to possible taste effects. Magnesium was chosen because it is known to be a net structure-maker and its double positive charge creates tenacious water-binding propensities and a corresponding negative contribution to apparent molar volume (-31cm^3 mol^{-1}). The use of magnesium hydroxide, rather than a magnesium salt at pH 7.0, avoids a concomitant positive contribution to partial molar volume from the anion. At pH 8.5 the magnesium concentration is less than 1.0% of the total concentration of sweetener (11.0-30.0% w/v) and thus contributes insignificantly to the measurable density. Hence, apparent specific volumes were unaltered by the presence of magnesium hydroxide. However, apparent molar isentropic compressibilities, measured by changes in ultrasound velocity showed sensitive differences in the presence of magnesium hydroxide. In all sweetener solutions, sucrose or sodium saccharin, the magnesium hydroxide elevates the apparent molar isentropic compressibility by up to 2.34 x$10^{-4}$$mol^{-1}$$bar^{-1}$ which means that the compactness of the hydration layer around the solute is not as great in the presence of magnesium hydroxide. Although apparent specific volume is better established as an indicator of taste quality than is apparent specific isentropic compressibility, the former may be relatively insensitive in experimental conditions such as these. Both parameters show similar trends since both are related to water-interactions and molecular packing characteristics. Elevation of apparent molar isentropic compressibility means that the solution approaches more closely to the open structure of pure water. The resulting water mobility advantage in the vicinity of ion-channels may thus explain the reports of certain cations as sweet taste enhancers.

3 Slide

COVARIATION IN HUMAN BITTERNESS PERCEPTION TO ELEVEN COMPOUNDS

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Human bitterness perception shows tremendous variation from person to person. As a function of correlations among individual sensitivities to bitter compounds, the number and variety of potential bitterness transduction systems may be inferred. Sensitivities to PROP (n-propylthiouracil) and PTC (phenylthiocarbamide), whose threshold-frequency distributions are described as bimodal (or trimodal), are the most commonly studied bitter compounds. To understand bitterness interrelationships, many attempts have been made to correlate sensitivities to PROP & PTC with other compounds, especially other bitter stimuli. In the present study, less frequently used classes of bitter compounds were employed, including at least one representative from several different chemical categories. Thirty-two subjects rated 11 compounds (quinine HCl, caffeine, (-)-epicatechin, tetralone®, l-phenylalanine, l-tryptophan, magnesium sulfate, urea, sucrose octaacetate (SOA), denatonium benzoate, and PROP) for bitterness and total intensity on the LMS scale, and repeatedly ranked 9 of these compounds (all but (-)-epicatechin and PROP), from weakest to strongest. The results indicate that ratings of PROP fail to correlate with those of the other 10 bitter stimuli, while ratings and rankings of these 10 compounds are correlated among themselves (e.g., tryptophan/phenylalanine/urea; SOA /caffeine; and denatonium/tetralone®). Principal components analyses of the ratings and ranks separate the compounds into at least two main clusters, neither of which contain PROP. This implies that, although there are close relationships among certain bitter compounds, PROP is detected independently from them. When subjects were grouped into the extremes of sensitivity to PROP (high, middle, and low), a significant difference was found in the bitterness ratings, but not the rankings, for these 10 compounds. This suggests there are small subsets of subjects who possess diminished or enhanced absolute sensitivity to all bitter stimuli, but do not differ in their relative sensitivities to these compounds.

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4 Slide

AQUEOUS-ETHANOL SOLUTION PROPERTIES OF CHLORHEXIDINE DIGLUCONATE

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The antimicrobial Chlorhexidine digluconate (CHXG) has it's level of inclusion in oralcare products limited by it's intense bitterness. The complete description of CHXG bitterness is not available, however hydration and packing characteristics of CHXG in water and in aqueous-ethanol milieu allow us to understand CHXG behaviour in the vicinity of the taste receptors.

Hydration and packing properties of CHXG in water, in aqueous-ethanol solutions and in mouthwash formulation were characterised at 20°C and 37°C in terms of (a) apparent specific volume - V2,and (b) isentropic apparent specific compressibility - K2(s)[Table 1].

Table 1: Solution properties of CHXG in water and in aqueous-ethanol solutions

	V2 (cm^3/g)[1]		K2(s) ($cm^3/g.bar$)[2]	
	20°C	37°C	20°C	37°C
1% CHXG + water	0.674	0.701	-1.16710-5	-2.68710-5
1% CHXG + 20% ethanol-water	0.680	0.803	-1.10210-5	-3.53610-5
1% CHXG + 40% ethanol-water	0.558	0.916	3.41310-5	7.8710-5
0.2% CHXG + 5% ethanol-water	0.690	0.73	-4.0 10-5	-2.0 10-5 (mouthwash formulation)

[1] V2 of bitter compounds lie within 0.71-0.93 cm3/g (Birch et al, 1993)

[2] K2(s)of bitter compounds lie within -2.5 x 10-5 - 3.0 x 10-6 cm3/g.bar(Parke & Birch, 1999)

CHXG is a hydrophobic molecule with good packing characteristics in water. Its hydration layer is not compact, showing little solute-water interactions. In ethanol-water,the interaction of CHXG with the solvent is highly dependent upon the solute concentration, and the ethanol has a strong influence on the packing structure of the solution. Increasing concentration CHXG increases the packing efficiency of the molecules in solution and counterbalances the effect of the ethanol in the mixture. Temperature rise increases apparent specific volume and isentropic compressibility due to the thermal expansion of the system. At 20°C, V2 and K2(s) values are borderline between the sweet and bitter range of taste quality, whereas at 37°C, they are indicating of CHXG molecules lying in the bitter range of the taste.

Birch, G.G et al.(1993)In Sweet-taste chemoreception. pp.129-139. Mathlouthi M et al (Eds.). London: Elsevier Appl. Sci.

Parke SA & Birch GG (1999)J.Agri.Food Chem., in preparation.

5 Slide

PSYCHOPHYSICAL INVESTIGATIONS OF IBUPROFEN: EFFECTS OF ORAL PH, BUFFERING, AND SALIVA

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The purpose of this study was to investigate the oral sensory properties of the non-steroidal anti-inflammatory drug ibuprofen. Anecdotal reports indicated that in solution ibuprofen produces both bitterness and throat irritation. We first measured gustatory and chemesthetic sensations in the mouth and throat after subjects swallowed ibuprofen solutions. Since previous work showed that salts can inhibit bitterness, we included conditions in which ibuprofen was mixed with bicarbonate salts. The results confirmed that ibuprofen primarily irritates the throat, and that in addition to the burning and stinging typical of other sensory irritants, most subjects reported distinct sensations of throat "tickle". The salts principally attenuated the overall intensity of throat irritation, although the incidence of tickle was not reduced. We hypothesize that the attenuation occurred because the salts elevated solution pH. Ibuprofen also differed from more typical irritants (e.g., capsaicin) in that repeated presentations led neither to sensitization nor desensitization, and its irritancy was independent of solution temperature. We investigated oral pH and buffering capacity as possible factors for the large individual differences in ibuprofen's irritancy. We found that the more neutral and more strongly buffered a solution was, the more irritation it caused. We further found that resting saliva buffered ibuprofen to near neutral pH (causing more irritation), whereas stimulated saliva buffered the solutions to a higher pH. The impact of resting saliva on ibuprofen's irritancy was successfully modeled by oral rinses with select organic buffers. We conclude that ibuprofen has psychophysical characteristics, perhaps shared by other NSAIDs, that are indicative of different excitatory mechanisms than those responsible for detection of better known sensory irritants such as capsaicin, and that these mechanisms depend at least partly on pH.

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6 Slide

THE TASTE OF FAT AND ITS METABOLIC IMPLICATIONS

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We have demonstrated that oral fat exposure results in a prolonged elevation of serum triacylglycerol (TAG) postprandially in humans. The present study sought to identify the chemosensory signal associated with modulation of lipid modulation. Seventeen healthy adults participated in four tests sessions conducted weekly. At each session, a fasting blood sample was collected followed by randomized administration of one of four treatment combinations. These included: ingestion of 50 one-gram capsules of safflower oil with 500ml of water in 15 minutes followed by oral (taste and odor) stimulation; load ingestion followed by orthonasal olfactory stimulation; no load with oral stimulation or no load with no sensory stimulation. Sensory stimulation entailed smelling or chewing and expectorating 5g samples of cream cheese on a cracker every 3 minutes for 60 minutes and every 15 minutes for an additional 60 minutes. Blood was drawn 2, 4, 6, and 8 hours post loading and analyzed for serum TAG. Following loading with oral stimulation, serum TAG was significantly elevated from baseline at hours 2, 4 and 6. Olfactory stimulation alone led to a significant elevation only at 4 hours. No load treatments led to progressively lower TAG. Peak TAG was significantly higher after loading with oral stimulation than all other treatments. Area under the curve (AUC) was significantly higher after oral stimulation plus load relative to all other conditions. The AUC for load plus olfactory stimulation was significantly higher than both non-load conditions. The greater TAG response to oral stimulation relative to odor alone suggests the effective stimulus was taste. The greater response with loading suggests the TAG rise is not attributable to de novo lipid synthesis. Enhanced lipid absorption and/or decreased clearance remain possible control sites. These data further support a gustatory component for dietary fat and its influence on postprandial lipid metabolism.

7 Slide

SUB-THRESHOLD INTEGRATION OF TASTE AND SMELL

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Central neural integration of sensory input from different modalities is critical for many types of perception and behavior. The perception that one has taken a bite from an apple, for instance, involves the cohesive integration of visual, somatosensory, olfactory, gustatory, and auditory cues into a unified experience. The perception of a flavor may be one of the best examples of such an integrative process, whereby activation in two peripherally-distinct, neural systems, olfaction and gustation, combines to give rise to a unitary oral sensation of flavor. Although animal studies have shown that certain neurons are uniquely responsive to combinations of odor and taste stimuli, behavioral evidence for an integration of odor and taste into flavor perception has only been found at suprathreshold levels. We utilized a novel psychophysical method to evaluate the joint contributions of odor and taste to the detectability of an olfactory stimulus. Using this method, we observed that the presence of an intra-oral, sub-threshold saccharin solution led to a reliable decrease (ranging from 13-57 %) in the threshold for benzaldehyde presented ortho-nasally. These results provide experimental evidence in humans of subthreshold, cross-modal chemosensory integration. In contrast, the presence of water or a solution of MSG did not decrease the benzaldehyde threshold, raising the possibility that the enhancement was facilitated by congruency between the specific odor and taste pairing. This finding implicates central loci of convergence for olfactory and gustatory information, and suggests the possibility that the enhancement in neural response to an odor-taste combination may reflect associations based on prior experience.

13 Poster

FEEDING RESPONSE OF THE MUD SNAIL *ILYANASSA OBSOLETA* TO SUCROSE AND DEXTROSE.

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Snail-feeding response to sugar is poorly studied. Experiments were done with different sugars to determine snail feeding and behavior. This experiment tested the mud snail *Ilyanassa obsoleta* for a preference between two sugars, dextrose and sucrose. A total of 24 snails were placed through a series of dextrose and sucrose concentrations. Behaviors and responses (number of snails rasping and number of rasps) were recorded. Although there was no significant difference between the percent of snails that either crawled out of the solution or remained in their shell for dextrose and sucrose, there were a significant number of snails that rasped while in dextrose. The mean number of rasps in dextrose was also greater than the mean number of rasps in sucrose. These results indicate that the mud snail *I. obsoleta* can distinguish dextrose from sucrose and that they have a preference for dextrose.

14 Poster

ACID ACCEPTANCE IN 28 MOUSE STRAINS

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The goal of this study was to characterize variation in acid acceptance among inbred mouse strains. Male 129/J, A/J, AKR/J, BALB/cByJ, BUB/BnJ, C3H/HeJ, C57BL/6J, C57L/J, CBA/J, CE/J, DBA/2J, FVB/NJ, I/LnJ, KK/H1J, LP/J, NOD/LtJ, NZB/B1NJ, P/J, PL/J, RBF/DnJ, RF/J, RIIIS/J, SJL/J, SM/J, SWR/J, SEA/GnJ, CAST/Ei and SPRET/Ei mice (n = 6 - 12 per strain) were obtained from The Jackson Laboratory and caged individually. Solutions of 0.01, 0.1, 1, 10 and 30 mM citric acid were presented in increasing order of concentration using 48-hr two-bottle tests, with one drinking tube containing the acid solution, and the other tube containing water. Most of the mouse strains were relatively indifferent to 0.01 - 1 mM citric acid and strongly avoided 10 and 30 mM solutions. Two strains, SEA/GnJ and SPRET/Ei, were notably more sensitive than the rest; they strongly avoided 1 mM and higher citric acid concentrations. Two other strains, C57L/J and NZB/B1NJ, were much less sensitive than the rest; they were indifferent to citric acid concentrations up to 10 mM and only moderately avoided the 30 mM solution. This study reveals strains of mice suitable for studying the genetic determinants of citric acid acceptance and perhaps sour taste.

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15 Poster

EFFECTS OF ICV NPY ADMINISTRATION ON SUCROSE TASTE REACTIVITY

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ICV neuropeptide Y (NPY) administration exerts a potent orexigenic effect. However, it is unclear whether NPY enhances intake via influences on taste evaluation. We therefore investigated the effect of 3V NPY infusion on taste reactivity. Rats fitted with 3V and intraoral cannulas were centrally injected with 5ug/5ul NPY or vehicle. Rats then received inter-digitated 50ul intraoral injections of 1.0 M and 0.1 M sucrose, one every 2 min for 48 min followed by three 50ul water rinses (the third rinse was used for analysis). Orofacial responses were scored for 3 ingestive consummatory acts: lateral tongue protrusions, medial tongue protrusions, and bout duration (1 sec pause criterion). These measures are regarded as a hallmarks of palatability (e.g., Grill et al., 1987). The internal control confirmed this supposition: all three measures were elevated with increases in sucrose concentration. NPY, however, exerted a specific effect compared to vehicle; medial tongue protrusions were elevated about 200% for both concentrations of sucrose, but not for water ($p < 0.0001$), and there was no effect on lateral tongue protrusions or burst duration. Therefore, NPY exerts a unique effect on taste reactivity that does not directly mimic the influence of taste per se. However, the effect was stimulus-specific — water trials were entirely unaffected. This suggests that NPY has some interaction with sensory processing. We are therefore evaluating 48 h food deprivation in this testing paradigm to assess whether this NPY effect mimics the state influence of natural deprivation. Supported by F32-DC00348, DC00417, DC00418

16 Poster

CHANGES IN THE RATE OF LICKING CONCENTRATED NACL SOLUTIONS DURING DIETARY NA⁺ DEPRIVATION PRECEDE INCREASED 24-HOUR DEPRIVATION-INDUCED NACL INTAKE

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Na^+ deprivation appears to alter the sensitivity of peripheral taste receptors to NaCl (Contreras and Frank; J. Gen. Physiol., 1979). Thus, the observation that increased intake of concentrated NaCl solutions by rats occurs only after 7-10 days of dietary Na^+ deprivation may be explained, in part, by a comparable delay in the change of the sensitivity of taste receptors. This study compared the time course of changes in psychophysical measures of taste responses to concentrated NaCl solutions during dietary Na^+ deprivation with the time course of the increase in 24-h intake of concentrated NaCl solution induced by Na^+ deprivation. Rats that had been trained to consume fluids rapidly during 10-s tests ($n = 11$) increased the rate of licking 0.5 M NaCl by 11.2 ± 5.5 licks/10 s after 2 d of dietary Na^+ deprivation. No further increase occurred after 5 or 10 d of Na^+ deprivation ($+15.5 \pm 3.3$ licks/10 s, $+12.4 \pm 4.4$ licks/10 s, respectively). In contrast, 24-h intakes of 0.5 M NaCl by rats after 2 d ($n = 8$) or 5 d ($n = 7$) of Na^+ deprivation did not differ substantially from baseline intakes ($+2.5 \pm 2.4$ ml, $+3.1 \pm 2.6$ ml, respectively), whereas intake after 10 d of Na^+ deprivation ($n = 8$) increased by 9.7 ± 3.4 ml. These results suggest that changes in gustatory-mediated responses do not underlie the delayed onset of Na^+ ingestion during dietary Na^+ deprivation. Alternatively, 24-h intake tests may lack the resolution to detect subtle differences in consumption of NaCl solutions during Na^+ deprivation. Supported by NIH Grant DC 02641

17 Poster

MICE SUPPRESS MALARIA INFECTION THROUGH VOLUNTARY INGESTION OF A BITTER CHEMOTHERAPY AGENT

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Rodents have evolved a variety of feeding strategies for maintaining physiological homeostasis. We examined the possibility that they can self-medicate against a potentially lethal parasitic infection. We used an inbred strain of mouse (BALB/c) and a malarial parasite (Plasmodium berghei) as our model system. We asked whether infected mice would voluntarily ingest a solution containing a "bitter"-tasting chemotherapy agent (chloroquine), and if so, whether they would benefit from doing so. Seven days after infecting the experimental mice with the parasites, we provided them a choice between two water bottles, one contained water and the other a 1 mM chloroquine solution. We monitored daily consumption from these bottles, and progression of the malaria infection by tracking changes in % parasitemia of red blood cells and mortality. We had two control groups: malaria-control mice had access to chloroquine but were not infected, and chloroquine-control mice were infected but did not have access to chloroquine. The experimental mice experienced significantly less parasitemia and mortality than did the chloroquine-control mice. The ability of the experimental mice to contain the malaria infection was related to the fact that approximately 20% of their fluid intake was from the chloroquine solution. We found, however, that this consumption of chloroquine was not related to the malaria infection because the malaria-control mice ingested statistically similar amounts. When we surveyed the literature, we discovered many other examples of apparently healthy mammals sampling a diverse range of "bitter" substances. We conclude that this habit of sampling different "bitter" substances may represent a generalized behavioral mechanism for chemoprophylaxis against parasitic infections and other illnesses.

18 Poster

THE EFFECTS OF TEMPERATURE CUES ON INGESTIVE BEHAVIOR IN THE RAT

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Although there is some evidence examining the effects of trigeminal stimulation on ingestive behavior in the rat, it is unclear whether an association between a trigeminal stimulus and the effects of an illness-inducing agent follows a similar neural pathway as in a conditioned taste aversion. Before this question can be answered, a trigeminal stimulus with no gustatory component needs to be identified. In our laboratory, we have developed an apparatus that controls the temperature of two presented fluid containers. Since it is assumed that water provides no gustatory stimulation, preference and avoidance behavior to water at different temperature levels was shown in a series of experiments. In Experiment 1, naïve rats were given a two-choice preference test between cold and warm water (i.e., cold water calibrated to 10°C; warm water, to 40°C). It was shown that rats preferred the cold water to the warm water on the basis of three types of measurements: total intake, time spent drinking, and number of drinking episodes. Since rats generally preferred cold water rather than warm water, the cold water was used as the target stimulus in a conditioned aversion paradigm. In this second experiment, rats injected with LiCl suppressed intake of the cold water when compared to saline-injected controls, who showed a general preference to the cold water. Finally, Experiment 3 examined whether an aversion to a cold stimulus would be retained when a gustatory cue was added to the cold water after conditioning. It was shown that rats suppressed their intake of cold saccharin after being conditioned to avoid cold water. These data not only support the idea that preference and avoidance behavior can be shown on the basis of trigeminal stimulation, but also that such stimulation may interact with other feeding-related stimuli, such as gustatory stimulation.

19 Poster

EVALUATIONS OF ATTRACTANTS AND REPELLANTS IN NORWAY RATS

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Rodents are frequently difficult to control with rodenticide baits for crop protection in agricultural ecosystems due to several factors (e.g. neophobia, unpalatable taste, sublethal rodenticide exposure). To counteract some of these factors, flavor agents and odor attractants can be used to ensure baits are consumed quickly when first encountered by problem rodents. Flavor agents can improve baiting efficacy, and can also improve control selectivity. Birds, reptiles, and small predatory mammals would be less likely to encounter the rodenticide if it is rapidly consumed by the target species. To simulate a field baiting model, two rectangular observation arenas (150 x 60 x 75 cm) with 2 choice compartments were constructed for observing the behavior of individual Norway rats. Preweighed quantities of Environmental Protection Agency (EPA) standard challenge bait mixture (65% corn meal, 25% ground oats, 5% powdered sugar, 5% corn oil) were used as a highly palatable bait base that would induce feeding without the need for food deprivation. Wistar strain albino rats were tested on several attractants (rat urine, preputial gland extract, and carbon disulfide) and a natural repellant odor (coyote urine). Bait intake levels and arena compartment choice behavior indicated that only the carbon disulfide at 10 ppm had an effect ($p < 0.05$) on EPA challenge bait consumption. Effects were compared with odor only present and with the agent incorporated directly into the mixture. A low level of 0.20% zinc phosphide rodenticide, when added to EPA challenge bait, was compared with and without the carbon disulfide attractant in separate groups of Wistar rats. Bait intakes increased in the presence of the attractant indicating the potential for improved baiting efficacy.

20 Poster

EFFECT OF PRIMARY AND SECONDARY REPELLENTS ON CONDITIONAL AVOIDANCE LEARNING IN EUROPEAN STARLINGS

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We conducted feeding and behavioral experiments on European starlings (Sturnus vulgaris) to compare the efficacy of primary and secondary repellents when the peripheral senses are by-passed. Such tests are critical to the determination of whether primary repellents can be rendered as effective as secondary repellents. In Experiment 1 the unconditional stimuli [propylene glycol (PG), a nontoxic carrier; methyl anthranilate (MA), a primary repellent; and 2 levels (2 mg/kg and 10 mg/kg) of methiocarb, a secondary repellent] were delivered via oral gavage and compared to controls (no treatment). The conditional stimulus was a visual cue, i.e., a colored food cup with vertical black and orange stripes, during training and 2-choice learning. Compared to controls, birds treated with 2 mg/kg of methiocarb reduced food consumption, but other treatments did not affect food consumption. Birds treated with MA responded with increased frequency and duration of irritation behaviors. Birds treated with methiocarb were immobilized. Although the data indicated increased irritation and illness, follow-up learning trials indicated that birds failed to associate the unconditional and conditional stimuli. Contrary to previously published reports, methiocarb failed to induce food aversion learning. We hypothesized that the birds were distracted by replacement of the standard cage door with a clear plexiglass door (to enhance video taping and analyses). We compared birds treated with PG, MA, and methiocarb (2 mg/kg) but did not replace the standard cage door. Birds treated with both MA ($p = 0.014$) and methiocarb avoided food associated with the conditional stimulus. Enteric delivery of a primary repellent can induce food avoidance learning as effectively as a secondary repellent. These findings pave the way for use of primary repellents in formulations in ways previously not considered. Converting primary repellents to secondary allows for maximum repellent effect, using suites of compounds with wider margins of environmental safety.

21 Poster

SPERMIATED MALE SEA LAMPREYS RELEASE A SEX PHEROMONE THAT FUNCTIONS AS AN ATTRACTANT FOR OVULATED FEMALE SEA LAMPREYS

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Behavioral studies have suggested that adult sea lampreys (*Petromyzon marinus*) release sex pheromones that influence the behavior of conspecifics, although the exact timing of the release and the functions of these pheromones are unknown. However, recent electrophysiological studies have shown that male sea lampreys release potent odorants for conspecifics during spermiation. To determine whether these odorants function as a sex pheromone we observed the behavioral responses of adult sea lampreys to these odors in both laboratory and field conditions. Our results confirm that spermiated male sea lampreys release a sex pheromone that influences the behavior of ovulated female sea lampreys. When placed in a two-choice maze, ovulated females spent more time in the side of the maze containing the spermiated male odor and showed increased searching or swimming activity in response to this odor. Also, in a natural spawning stream ovulated females located and swam to cages containing spermiated males. We conclude that spermiated male sea lampreys release a sex pheromone that attracts ovulated female sea lampreys and functions to synchronize spawning behavior between ripe males and females. The Great Lakes Fishery Commission supported this study.

22 Poster

INDIVIDUAL COMPONENTS OF THE GOLDFISH PRE-OVULATORY PHEROMONE ELICIT DIFFERENT BEHAVIORAL RESPONSES: A FIRST STEP TO UNDERSTANDING THE ROLE OF MIXTURES IN A VERTEBRATE PHEROMONE

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Fish sex pheromones are the best understood among the vertebrates and are crucial for synchronizing reproduction. In the goldfish, pheromones are unspecialized hormonal products that are released throughout the reproductive cycle in varying ratios that change with reproductive status. Our long-term objective is to address whether the goldfish can obtain precise information about reproductive condition by discriminating specific hormonal compounds and their blends. In order to address this question we are examining the pre-ovulatory pheromone, which is composed of three steroids (17α,20β-dihydroxy-4-pregnen-3-one [17,20β P], 17α ,20β-dihydroxy-4-pregnen-3-one-20-sulfate [17,20β P-S], and androstenedione [AD]). As a first step this study examined the behavioral responses of male goldfish exposed individually to each of the three steroids. Groups of 3 males were observed for a 10-minute control period in aquaria. Fish were then observed during exposure to a steroid (10^{-9} Molar) over a 2-hour period. Behaviors observed included activity, chasing and nudging (reproductive behaviors), and pushing (an aggressive behavior). Each steroid elicited a different set of behaviors ($n = 12$ trials per steroid). 17,20β P, which is released by pre-ovulatory females prior to spawning, elicited a moderate increase in chasing and nudging that persisted throughout the experiment ($p < 0.05$). Exposure to 17,20β P-S, which is released by ovulatory spawning females in urinary pulses, elicited a large increase in nudging and chasing that lasted for only the initial 5 minutes ($p < 0.05$). In contrast, AD, which is released by both pre-ovulatory females and by mature males, elicited increases in aggressive behavior ($p < 0.05$). These results demonstrate that each component of the steroidal pheromone blend is discriminated separately and serves to elicit a distinctive pattern of behavior. Ongoing experiments are now examining whether differing ratios of components within these blends have effects on male behavior and endocrinology.

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23 Poster

INITIAL STUDIES ON THE SOURCE AND CYCLIC RELEASE PATTERN OF (Z)-7-DODECENYL ACETATE, THE PREOVULATORY PHEROMONE OF FEMALE ASIAN ELEPHANTS.

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Utilizing two complimentary headspace techniques, solid phase microextraction (SPME) and evacuated canister capture followed by cryogenic trapping (ECC/CT), prior to gas chromatography/mass spectrometry (GC/MS) , we have identified (Z)-7-dodecenyl acetate (Z7-12:Ac) in preovulatory serum of the Asian elephant and have established a semi-quantitative pattern for its presence in urine. These patterns are coincident with observed male behaviors and are consistent with biochemical and binding properties of the active ligand. Using SPME followed by GC/MS, Z7-12:Ac was measured in the headspace of native and protease-treated preovulatory serum. Using SPME and ECC/CT followed by GC/MS on duplicate aliquots of native urine of known pH, Z7-12:Ac was quantified throughout the estrous cycle. Follicular and luteal phases of the estrous cycle were confirmed by serum progesterone concentrations and by male and female behavioral indicators. Our molecular biology studies have demonstrated optimal binding of Z7-12:Ac at alkaline pHs. Therefore, aliquots of the same urine specimens were acidified, and the amount of Z7-12:Ac released from its protein carrier was measured. The amount of Z7-12:Ac was compared in fresh urine, urine stored frozen at -80oC and urine stored frozen at -20oC. Diminished amounts of acetate and increased levels of (Z)-7-dodecenyl alcohol were observed in the -20oC stored samples. Ongoing studies are aimed at quantitating serum levels and urine concentrations of both Z7-12:Ac and its degradation products, and assessing possible Z7-12:Ac in follicular stage cervical mucous samples. Of special interest is whether urinary and/or mucoidal Z7-12:Ac concentrations correlate with the dramatically increased clitoris-to-air tail flicking behavior by female elephants during the follicular period.

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24 Poster

URINARY AND TRUNK MUCUS PROTEIN CARRIERS OF (Z)-7-DODECENYL ACETATE, THE SEX PHEROMONE OF THE ASIAN ELEPHANT

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An in-depth investigation of the proteins involved in transport and recognition of the female-produced sex pheromone, (Z)-7-dodecenyl acetate (Z7-12:Ac), in the Asian elephant (*Elephas maximus*) is described. Utilizing a radiolabeled photoactivatable analog, [$^3H_2$]-(Z)-7-dodecenyl diazoacetate, we have identified a 66 kDa protein as the main urinary pheromone carrier. N-terminal sequencing revealed a strong homology to known serum albumins. Using RT-PCR, the full cDNA sequence of the elephant albumin was elucidated. Bioassays demonstrated that a semi-purified urinary albumin enhanced the bioresponses of males to Z7-12:Ac. Binding experiments with the elephant albumin have shown a strong dependence of pheromone binding on pH, with maximum in the range 8-10.

The male response to the urinary pheromone involves transport of the pheromone toward the vomeronasal organ (VNO). The urinary pheromone mixes with trunk mucus; this mixture is placed onto the mucus-laden incisive ducts leading to the VNO. Photoaffinity labeling allowed us to identify two closely related trunk mucus proteins homologous to known odorant binding proteins (OBPs) that bind the pheromone. Using antibodies against the elephant OBPs, tissues producing the proteins were identified, and cDNA cloning is in progress. The OBPs bind the pheromone with higher affinity than the urinary albumin, with low discrimination between various lipophilic ligands. The binding properties vary only little with changes in pH.

These results suggest that the pH difference between the urine and the trunk mucus may cause release of the pheromone from the urinary carrier protein, making it available for binding by the mucosal proteins. This phenomenon would effectively cause the pheromone concentration in the sensory organs to increase rapidly, rather than gradually. The overall effect is a significant increase of detection sensitivity, as observed in our behavioral studies.

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25 Poster

SILEFRIN, A FEMALE-ATTRACTING PHEROMONE IN THE ABDOMINAL GLAND OF THE SWORD-TAILED NEWT, *CYNOPS ENSICAUDA.*

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Sodefrin is a female-attracting peptide pheromone discovered in the abdominal gland of the male red-bellied newt, *Cynops pyrrhogaster.* Sodefrin-like pheromone was purified from the abdominal glands of congeneric species of the newts, *C. ensicauda* by gel filtration chromatography and reversed-phase HPLC. The final product comprised 10 aminoacids with the sequence SILSKDAQLK, a variant form of sodefrin with aminoacid sequence SIPSKDALLK. Both native and synthetic peptides had a prominent activity in attracting conspecific females. This sodefrin-like peptide was designated silefrin (sil represents N-terminal 3 aminoacids of this peptide). Immunohistochemical analysis revealed that silefrin existed exclusively in the epithelial cells of the abdominal gland of *C. ensicauda.* Furthermore, *in situ* hybridization and northern blot analysis using silefrin precursor cDNA as a probe revealed that silefrin precursor mRNA was expressed in the epithelial cells of the abdominal gland and that a combination of prolactin and testosterone enhanced the expression of silefrin precursor mRNA.

26 Poster

COURTSHIP PHEROMONE EFFECTS ON FEMALE RECEPTIVITY IN PLETHODONTID SALAMANDERS

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In many species of terrestrial salamanders (Family: Plethodontidae), males produce courtship pheromones that influence female receptivity and therefore male courtship success. Here, we report biochemical and behavioral analyses of courtship pheromones for the terrestrial salamander, Plethodon jordani.

Biochemical analyses reveal that glandular extracts are composed of proteins, with two proteins comprising about 85% of the total protein. Both of these two components, a 22 kDa and 10 kDa protein, exist in five or more isoforms and vary in the proportion of isoforms within and among populations.

Behavioral bioassays were used to test female responsiveness to a purified solution of one of these proteins, the 22 kDa protein. Courtship encounters were staged in which we experimentally delivered the 22 kDa protein (or saline control) to the female via a micropipette. In all experiments, the mental gland was ablated from each male to prevent uncontrolled pheromone delivery. Behavioral experiments revealed that the 22 kDa protein alone was effective at increasing female receptivity.

We discuss these results in the context of pheromone evolution.

27 Poster

PREDATOR ODORS AND THEIR EFFECTS ON THE REPRODUCTION SUCCESS OF PHODOPUS HAMSTERS

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Predator odors are known to influence the feeding behavior of several herbivore species. In contrast to the many studies on feeding inhibition in prey species due to predator odors only few studies have tried to estimate the effects of predator odors on the development and reproductive physiology of their potential prey. The present study focuses on the influence of predator (cat, ferret) urinary chemosignals on the reproductive success in Phodopus campbelli, a small hamster species native to Mongolia. In predator naive females exposed to the urine odor of a predator estrous cycles were severely disturbed: ovulations were delayed or even inhibited; litter sizes were smaller, sex ratio changed. Predator odors also had significant effects on the postnatal development and fertility in Phodopus males: The exposure to urine odor resulted in reduced postnatal weights of the testes and the epididymis. In addition testosterone levels were decreased by about 50% (RIA studies), and meiotic anomalies in the chromosomes could be detected (EM studies). Such asynaptic autosomal configurations were found in 34.9% of the investigated cells (exposed animals) while in control animals non were visible. However, the underlying mechanisms are not known. Also not known are the active component or components in the predator urine. Some authors contribute these effects to sulphurous components or their metabolites in predator urine.

28 Poster

HORMONAL MECHANISMS OF LITTER REDUCTIONS IN RODENTS UNDER PREDATOR ODOR INFLUENCE

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We used Norway rats as a model for potential prey and the urine of feral domestic cat maintained on a wild mouse diet as the test stimulus. Our earlier studies indicated that exposure to predator urine maximally affected implantation and maintenance of implantation when predator urine was applied to the bedding of rodents during the first third of gestation. We monitored progesterone levels in female Norway rats during early gestation because this a key ovarian hormone responsible for maintenance of the fertilized egg, preparation of the endometrium, and maintenance of pregnancy.At the same time corticosterone patterns were recorded for the same animals. Additionaly, rough handling group was used as a control for stress induced changes of plasma corticosterone level. As we observed in our previous studies, female rats exposed to cat urine had smaller litter sizes. Based on physical appearance of corpora leuteal scarring, it appeared that reduction in litter size was owing to resorption of the embryos during the early part of gestation. Consistent with the morphological evidence was the observation that plasma progesterone levels were dramatically suppressed in rats exposed to cat urine relative to levels observed in the water control group and for rats exposed to guinea pig urine. We did not observe statistically significant differences of plasma corticosterone levels for rats exposed to predator and non-predator urine, while rough handling of animals caused clear elevation of corticosterone. Rough handling did not cause reductions in litter size. This findings indicate that predator odors may work as specific reproductive disrupters. Evolutionary advantages are discussed.

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29 Poster

IMPROVED ODORANT DISCRIMINATION IN AN ARTIFICIAL NOSE THROUGH FEEDBACK CONTROL OF ENVIRONMENTAL SAMPLING

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We are developing an artificial nose for rapid sampling of volatile chemicals in the environment (AChemS XXI, 1999). The device presents odor samples to an array of optically-based, cross-reactive chemical sensors via brief negative pressure pulses ("sniffing"). Unknown test odors are identified by comparing sensor signals to a stored set of target signals. As in the biological olfactory system, sensors produce signals with different amplitudes and time courses for different odors. Furthermore, sampling parameters (e.g., sniff duration, amplifier gain, and various sensor control functions) also affect signal amplitude and time course. While one set of sampling parameters may produce discriminable signals for some odors, a different set of parameters may be optimal for other odors. A single sniff using one set of parameters may not be optimal for all odors. In addition, sampling parameters are often constrained by other factors that can limit odor signal amplitude. For example, brief sniffs are desirable in order to reduce sensor saturation so that samples may be acquired frequently. Brief sniffs, however, produce small odor signals, which degrades discrimination. To improve odorant discrimination given these various constraints, we have devised a method of sampling whereby signals produced by a sniff are analyzed and the results of that analysis are used to alter the sampling parameters of subsequent sniffs. This approach is inspired in part by the sniffing behavior exhibited by animals during odor sampling tasks. We have implemented this strategy in our device to improve brief sniff performance: a second, longer sniff is acquired if discrimination based on the brief sniff is poor. Signals produced by the longer sniff lead to marked improvement in discrimination performance. By altering sampling parameters in combination, it may be possible for the artificial nose to "learn" the optimal set of parameters for discriminating a given set of odors.

Supported by NIDCD, ONR, and DARPA.

30 Poster

GENETICALLY DETERMINED BODY ODORS EVOKE DISTINCT PATTERNS OF NEURAL ACTIVITY IN THE MAIN OLFACTORY BULB

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Individual identity can be discerned by many sensory systems. Within a given mammalian species, individuals can be identified by their unique body odors (odortypes). Evidence indicates that these odortypes may be determined by allelic differences at the major histocompatibility complex (MHC or H-2), X, and Y- chromosomes. Previous work on MHC-determined odortypes has shown that female mice(H-2^d haplotype) can discriminate between allelic differences found in the urines of congenic male mice (H-2^k versus H-2^b haplotypes). We have utilized this model system to test whether odortypes elicit unique maps of neuronal activity in the main olfactory bulb (MOB). Such distributed patterns of odor-induced neuronal activity likely contribute to the encoding of odortype information. To compare odor representations elicited by these odortypes, we constructed maps of c-fos mRNA expression in the MOB. Female H-2^d mice were exposed to urine odors from H-2^k or H-2^b congenic male mice. Both urine odors elicited highly distributed patterns of activity throughout the glomerular layer. H-2^k and H-2^b urine odors evoked distinct activity maps. While H-2^k urine odor activated a large area ventrally, H-2^b activated a more restricted area within this region. Portions of these regions were shown to be statistically different by Chi Square and Mann-Whitney analysis at significance levels $p < 10^{-4}$. In addition, roughly half of the animals showed punctate regions of activity in the dorsomedial region of the MOB evoked by H-2^k but not H-2^b urine odor. Commonly activated regions were found throughout the rostrocaudal extent in ventral and dorsolateral regions of the MOB. These results show that MHC-determined odortypes elicit different spatial maps of neural activity within the MOB. Thus, we present the first anatomical and functional evidence that odortypes can be encoded by distinct spatial patterns of glomerular activation in the MOB.

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31 Poster

RESPONSES TO OLFACTORY AND INTRANASAL TRIGEMINAL STIMULI: RELATION TO THE RESPIRATORY CYCLE

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The aim of the study was to investigate whether the perception of intranasal chemosensory stimuli changes in relation to the respiratory cycle. We investigated 40 healthy subjects with normal olfactory function. They participated in 4 sessions (20 women, 20 men, age range 19-39 years, mean age 23 years). The first session was used to adapt subjects to experimental conditions, and, specifically, to train a certain breathing technique (velopharyngeal closure) preventing intranasal respiratory air-flow. In each of the following sessions one of three stimulants was tested, namely phenyl ethyl alcohol (25% v/v), hydrogen sulfide (2 ppm), or the trigeminal stimulant carbon dioxide (50% v/v). The sequence of testing the stimulants was randomized. Sessions were separated by at least one day. Chemosensory event-related potentials (ERP) were recorded in response to 80 stimuli each (mean interval 30 s, stimulus 200 ms). Following each stimulus subjects rated its intensity using a computerized visual analogue scale. Respiration was recorded using a probe in front of the subjects' mouth. While presentation of chemosensory stimuli was performed independent of the respiratory cycle, responses were averaged off-line according to the subjects' respiratory phase when the stimuli had been presented. Perceived intensity of olfactory or trigeminal stimuli did not differ in relation to the respiratory cycle ($F[2,68]>2.61$, $p>0.097$). Olfactory ERP to PEA were larger for inspiratory stimuli (N1: $F[1,38] = 4.96$, $p = 0.032$; P1N1: $F[1,38] = 4.40$, $p = 0.043$; N1P3[1,38] = 10.1, $p = 0.003$). Similar findings were made for H2S (N1: $F[1,35] = 3.97$, $p = 0.054$). In addition, responses to CO2 were larger when stimuli were presented during inspiration (N1: $F[1,37] = 6.94$, $p = 0.012$). In general, differences in relation to the respiratory cycle were found for early ERP components, while they were less pronounced for late components. These data indicate on an electrophysiological level that there is priming of both, olfactory and trigeminally mediated sensations in relation to the respiratory cycle.

32 Poster

ODOR-SPECIFIC REGIONAL ACTIVATION OF RAT PIRIFORM CORTEX

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Previous work has demonstrated a selective activation of small groups of glomeruli in the olfactory bulb (OB) in response to odorants, but the extent to which such spatial organization exists in piriform cortex (PC) remains unknown. Although afferent and intrinsic connections of PC are highly distributed spatially, there is evidence that afferent input to the anterior part of piriform cortex (APC) may be concentrated in patches (Ojima et al., 1984; Buonviso et al., 1991). To determine if there is a function-related spatial organization in PC, we used cellular-level immunocytochemical localization of Fos protein following exposure to single odorants. Male hooded rats (250-350g) were placed in a clean cage for 18-24 h, then intermittently exposed to odor for 30 s separated by 90 s intervals for one hour and rapidly perfused with fixative. Littermate control rats were treated identically, but without exposure to odorants. Results from odor-exposed animals showed that activity in response to pure compounds was concentrated in patches within APC. Moreover, the activity in APC evoked by chemically disparate odors occurred in spatially distinct but overlapping patches. In posterior piriform cortex (PPC), a higher number of cells displayed Fos reactivity, and spatial patterns were much larger and ill-defined. Control animals exhibited very low levels of Fos labeling. These results provide evidence that response specificity in APC is spatially organized, whereas activity in the PPC appears to be more widespread. This suggests a progressive transition from a precise modular representation of odor quality in OB to a spatially distributed ensemble code in PPC.

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33 Poster

ENSEMBLE CODES FOR DYNAMIC OLFACTORY STIMULI RECORDED WITH MULTICHANNEL SILICON MICROPROBES IN THE MOTH ANTENNAL LOBE

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Over many decades, numerous reports have provided sound evidence that precise spatial and/or temporal codes are involved in the recognition and discrimination of odors in ensembles of olfactory neurons at the first stages of processing in the CNS. Some studies have also suggested that temporal activity patterns change in an "odor-specific" manner. For this to be true, however, it must be shown that the precision of an odor-evoked activity pattern is maintained in situations when the stimulus itself is temporally complex, and odor concentrations are changing on a rapid time scale, as typically occurs in nature. To address this question, we recorded ensemble activity from the moth antennal lobe using a 3-pronged, multichannel silicon microelectrode array. Following the recordings, the patterns of ensemble activity were localized to specific olfactory glomeruli through morphological identification of the 3 probe tracks and their multiple recording sites. In accordance with our single-unit data, temporal analysis of ensemble patterns revealed that the timing of synchronous firing among antennal lobe neurons is unpredictable, and varies with the time-course of a changing odor stimulus. While oscillations are a prominent component of moth olfactory network dynamics, we found no evidence that the patterns of odor-evoked spiking in glomerular projection neurons (PNs) are constrained by oscillations, as shown in other insects. In this olfactory system, therefore, odor-specific information is not encoded in the temporal precision of spike discharges in glomerular PNs. Instead, chemical identity is represented as a spatial code, according to which glomeruli are activated (and/or inhibited), and necessary information about stimulus concentration and dynamics is encoded in the temporal sequences of spikes relayed to higher brain centers.

Supported by DARPA

34 Poster

TRANSIENT SYNCHRONIZATION OF GLOMERULAR OUTPUT NEURONS IS MODULATED BY ODOR DYNAMICS IN THE MOTH ANTENNAL LOBE

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Synchronous firing among neurons is widely believed to help integrate distributed but related signals in a neural network, thus creating a stronger, more coherent representation of a given stimulus in the brain. In the olfactory system, recent studies of glomerular projection neurons (PNs) provide evidence for odor-evoked oscillatory synchronization of PN ensembles in several insect species. We wanted to know if PNs in the moth antennal lobe are also synchronized by odor stimulation, and whether synchrony is governed by oscillations or by other mechanisms. Simultaneous intracellular recordings were obtained from pairs of PNs innervating one or more glomeruli of the macroglomerular complex (MGC) in male moths. A diverse population of MGC-PNs transmits information about the female sex pheromone to specific centers in the protocerebum for further processing. Thus, the MGC serves as an excellent model to investigate the timing relationships among populations of functionally related neurons. Temporal analysis of odor-driven response trains revealed that spikes in PNs tuned to the same stimulus were more tightly correlated than those responding to different inputs. Dynamic correlation analysis (sliding window = 5 msec), however, failed to reveal any periodic pattern of synchrony among PNs, which would be expected if synchrony were modulated by an underlying oscillation. Instead, synchrony was tightly correlated to odor onset, even in multiple-pulse stimulus trials. Some PN pairs also were synchronized at stimulus offset, but this appeared much less frequently. Inhibitory potentials (IPSPs) often occurred at the onset of each odor response, and thus a shunting mechanism may aid in the temporal precision of onset synchronization. Our results indicate that in the moth antennal lobe, MGC-PN synchronization is not constrained by oscillations. Instead, timing remains flexible, allowing synchronized MGC-PN spike trains to be modulated from moment to moment to reflect input from naturally dynamic olfactory signals.

Supported by DARPA

35 Poster

OLFACTORY CONDITIONING IN *MANDUCA SEXTA*: EVOLUTION OF NEURAL ENSEMBLE PATTERNS IN THE ANTENNAL LOBE BEFORE, DURING, AND AFTER LEARNED ASSOCIATION TO ODORS

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Recent experiments demonstrate that the sphinx moth (*Manduca sexta*) can be trained in classical and discrimination conditioning paradigms to associate odors with a food reward. We have now developed a physiological preparation that allows us to investigate patterns of neural ensemble activity in the antennal lobe (AL) before, during and after olfactory conditioning. Using multichannel silicon microprobes in the AL, and cibarial pump (CP) activity to measure the conditioned response, we have observed patterns of ensemble activity in the AL that evolve during training and gradually stabilize following the conditioning process. Patterns at the onset of training are generally simple, involving only one or a few neurons. After only a few training trials, distinct changes in the patterns of ensemble activity were observed. These changes included new cells joining the ensemble, or suppression of cells that were previously active. About 90 min after training began, we tested for conditioned responses in the absence of reinforcement. As these tests were repeated, the ensemble patterns correlated to the CP response showed further changes. For example, a progressive increase in spike activity in one cell was correlated with a progressive delay of spike activity in another cell. While the basic pattern was repeated with continued testing, the timing of these activity sequences grew more precise over the next several hours, suggesting that these specific cellular interactions became stronger over time. This stabilization process could thus reflect a mechanism for memory consolidation at this early stage of olfactory processing. Importantly, these odor-evoked activity patterns were not constrained by an oscillation, nor was there a unique and reproducible pattern of ensemble activity that encoded information about odor identity. Rather, the initial odor representations evolved with time in the context of acquiring and retaining learned associations to odor.

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36 Poster

NITRIC OXIDE AFFECTS SYNAPTIC EFFICACY IN THE ANTENNAL LOBE OF *MANDUCA SEXTA*

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We are examining the role of the NO/sGC pathway in the processing of olfactory information in the antennal lobe of the hawkmoth, *Manduca Sexta.* We found that soluble guanylyl cyclase (sGC) is highly expressed in a subset of antennal lobe neurons and that nitric oxide synthase (NOS), the enzyme that generates nitric oxide (NO), is expressed in the axons of apparently all olfactory receptor neurons. These expression patterns suggest the possibility that odorant stimulation of olfactory receptor neurons causes the release of NO and that this phenomenon plays an important role in the subsequent processing of that odor signal. We are testing this hypothesis in two ways. First, we are using the NO-sensitive dye Daf-2DA to visualize NO in the antennal lobes. Second, we are recording from antennal lobe neurons using both single and multi-unit recording methods before and after treatment with agonists and antagonists of NO pathways.

Using Daf-2DA, we found labeling of the antennal lobe cell bodies and the glial cells in the antennal nerve and antennal lobe in the absence of any exogenous treatment. This staining was eliminated by preincubation with the NOS inhibitor, L-NAME. Direct electrical stimulation of the antennal nerve resulted in an increase in fluorescence in the caps of some glomeruli.

Using single-unit responses of antennal lobe neurons, we found that agents that interfered with NO signaling, including L-NAME and Carboxy-PTIO, caused a dramatic and reversible change in response latency and a desynchronization of the response to repeated stimulation. SNP, an NO donor, caused a dose-dependent depolarization of antennal lobe neurons. The phosphodiesterase inhibitor IBMX, an agent that should potentiate the NO/sGC response, caused depolarization of the cells and also blocked spiking. These data point to an important role for the NO/sGC signaling system in maintaining synaptic efficacy in the olfactory pathway.

37 Poster

INHIBITION IS A MAJOR NEURAL ACTION COMMUNICATED TO THE CRAYFISH OLFACTORY FOREBRAIN BY ACCESSORY LOBE PROJECTION NEURONS IN RESPONSE TO ANTENNULAR STIMULATION.

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In crustaceans, olfactory receptor neurons terminate within an olfactory lobe (OL), where primary neural processing of odor information occurs, and from where processed information is passed to the forebrain. Odor information also is believed to pass, via interneurons, to the adjacent accessory lobe (AL) in the midbrain. The major output pathway of both the OL and the AL are axons of projection neurons (PNs) having somata in cell cluster 10. At least three classes of PNs are present: those with dendritic arborizations solely in the OL, those that arborize only in the AL, and those in which arborizations occur in both the OL and the AL. PN axons from both the OL and AL course within the olfactory-globular tract to the lateral forebrain, or medulla terminalis (MT), in the eyecups. One target of the PNs within the MT is the hemi-ellipsoid body. Local interneurons (LPIs) within the hemi-ellipsoid body generate EPSPs and delayed impulse bursts following exposure of the antennules to odors, and it is inferred that this is from activity in those PNs having dendrites arborizing within the OL. Recent evidence obtained from focal electrical stimulation indicates that LPIs also receive both brief excitation and feed-forward inhibition from PNs that arborize within the AL. In fact, excitation followed by secondary inhibition appears to be a consequence to stimulation of the antennules with either electrical shocks or pulses of odorant, suggesting that responses in AL projection neurons are evoked by such input in addition to the activity evoked in OL projection neurons. Focal stimulation of a neuron cluster at the base of the hemi-ellipsoid body, and antiGABA-like immunostaining both suggest that the inhibitory neurons reside locally within the MT and that they may be directly excited by AL projection neuron activity. Supported by grants from NSF and from NIDCD.

38 Poster

IDENTIFIED PHEROMONES EVOKE DISTINCTIVE SPATIAL MAPS OF ACTIVITY THAT ARE INDEPENDENT OF CONCENTRATION IN THE GOLDFISH OLFACTORY BULB.

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Pheromones are organismal odors that evoke stereotypic and innate responses in conspecifics. As such, they are excellent tools to explore how the olfactory system discriminates natural odors. Five pheromonal components have been identified in goldfish and we use them here to test if biologically relevant odorants evoke distinct spatial patterns of activity in the vertebrate olfactory bulb. Field potentials were recorded from the dorsal olfactory bulb of male goldfish over a twelve-point grid. Three components of the pre-ovulatory pheromone (sex steroids), two components of the post-ovulatory pheromone (F-prostaglandins), and controls were initially tested at a single, biologically relevant concentration (sub-micromolar). Once spatial maps were established, the effects of odorant concentration on these maps were examined. Data were analyzed using a novel form of time series analysis. Pheromones elicited oscillatory responses with characteristics similar to those evoked by amino acids and bile acids in other studies. Each pheromonal odorant evoked a unique spatial map. For example, 15-keto prostaglandin F2α (15K) evoked activity with a peak in the lateral bulb (peak position showed responses in four of eight trials) and 17a ,20b -dihydroxy-4-pregnen-3-one-20-sulfate (1720b P-S) evoked activity with a peak in the medial bulb (peak position showed responses in four of eight trials). Concentration did not influence the fundamental structure of these maps (n = 6). For example, 1720b P-S evoked similar spatial maps when tested at 10^{-9}M and 10^{-8}M. Finally, to confirm our field potential results, we conducted single-unit recordings from bulbar projection neurons in the 15K and 1720b P-S 'hot spots'. As in the field potential recordings, four of eight trials in each location showed excitation to the appropriate pheromones (F-prostaglandins and sex steroids). In conclusion, this study suggests that spatial distribution of neural activity plays an important role in encoding information from biologically relevant odorants in the vertebrate olfactory bulb.

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39 Poster

SPATIAL PATTERNS OF OLFACTORY BULBAR RESPONSES TO PUTATIVE ODORANTS IN A MARINE TELEOST

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In vertebrates, the olfactory bulb is a primary center processing olfactory information. It is as a convenient part of the brain for studies aimed at unraveling the underlying principles of neuronal processing in central nervous system. Our previous work as well as other studies on the olfactory system of red sea bream, Pagrus major have not only demonstrated a well_developed olfactory organ and olfactory brain, but electrophysiological studies have shown that amino acids are highly stimulatory to the olfactory system rendering red sea bream a suitable model for neurophysiological investigation. A number of techniques have demonstrated that putative odorants elicited patterns of neuronal activity that are distributed across cells of the vertebrate olfactory epithelium (Thommesen and Doving, 1977, Acta Physiol. Scand., 99: 270_280) and olfactory bulb (Cinelli et. al., 1995, J. Neurophysiol., 73: 2053_2071). We further examined the spatial patterns of neuronal activity at the olfactory bulb by recording the electroencephalographic responses (EEG) elicited by amino acids, a bile acid and a natural odorant. Induced responses to the amino acids and to the natural odorant recorded at medial, central and lateral bulbar regions are remarkably similar in magnitude except that the responses obtained from the central bulbar area are comparatively reduced. An almost negligible difference in magnitude between the amino acids and the natural odorant responses was observed in the first 8 seconds and 20 seconds of odorant stimulation when compared with the total area of integrated EEG. The bile acid failed to elicit any response at the three bulbar regions. These data suggest that encoding of amino acids and the natural odorant is processed by a wider region of the olfactory bulb while the olfactory signals due to the bile acid are processed in a fashion distinct from those of the amino acids/natural odorant in the red sea bream.

40 Poster

CHANGES IN SPATIO-TEMPORAL PROPERTIES OF ODOR RESPONSES FROM MULTIPLE ODOR PRESENTATIONS IN THE TURTLE BULB

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We made voltage-sensitive dye measurements of the response to several odorants in an in vivo turtle preparation. The turtles were anesthetized and craniotomy was performed over the olfactory bulb. The bulb was stained with 0.1 mg/ml solution of the styryl dye RH414. We measured the optical signals with a 464 element photo-diode array.

Four different population signals to a single odor stimuli were detected: a DC response and three oscillations (rostral, middle and caudal). Those oscillations had different spatio-temporal properties (location, frequency and latency).

We applied multiple odor presentations with different inter-stimulus intervals (ISI). The oscillatory response to the consecutive stimuli was different depending on the ISI: 1) if ISI was below 2s all components of the responses to the second stimulus were greatly reduced; 2)if ISI was above 2s and below 11s the rostral oscillation disappeared and the caudal oscillation approximately doubled its frequency; 3) if ISI was above 11s the response to the second stimulus was the same as to the first. The spatial position of the components that were present was apparently the same during both stimuli. The dramatic change in the response to moderate ISI's suggests that the oscillations represent higher order processing that depends on the context of the stimulus.

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41 Slide

DETERMINANTS OF ACTIVITY FOR ALDEHYDES AT THE MAMMALIAN OCTANAL RECEPTOR I7

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To further understand the structure-activity relation between odorant and receptor, we have begun to investigate the molecular determinants of activity for different aldehydes at the octanal receptor, OR-I7. Several aldehydes (C5-C12 range) with different degrees of unsaturation and/or branching and substitutions were investigated for their activity. We show that aldehydes but not nitrile, thiol or other functional groups activate the receptor. Moreover, the OR-I7, besides being activated by the saturated C7-C11 aldehydes, is also activated by unsaturated and branched aldehydes in the same size range. Thus, cis-6-nonenal and citronellal, a branched and unsaturated aldehyde, were similar in activity to octanal. Interestingly, both isomers of citronellal and a cyclohexyl aldehyde were effective yet all the benzyl aldehydes investigated, as well as hydroxycitronellal, were ineffective. These results suggest that although the binding pocket may accommodate a side chain with various degrees of unsaturation and branching, it may not allow interaction with the π system of a benzyl group, and it may not accept a polar substitution in the side chain. The activity of all the aldehydes correlated with the length of the molecule, with an optimal length for activity centered at about 9.2 A°. We also found that the activity of the aldehydes is particularly sensitive to modifications (methylation and unsaturation) at carbon 2 (c2) and 3 (c3). Molecules with a methyl group at c2 were inactive, and citral, a citronellal analog, with unsaturation at c2 and methylation at c3 was inactive. Taken altogether these results indicate that there are at least 3 determinants of activity for aldehydes at the OR-I7: the critical presence of an aldehyde group, an optimal molecule length, and steric restrictions imposed by carbons in the vicinity of the aldehyde group.

42 Slide

BEHAVIORAL AND OPTICALLY RECORDED MUCOSAL OLFACTORY ACTIVITY PATTERNS IN RESPONSE TO AN HOMOLOGOUS SERIES OF ALDEHYDES IN THE RAT

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To further understand both the relationship of odorant-induced activity patterns to the reported zonal distribution of olfactory receptors and whether the odorant-specific activity patterns recorded from the mucosa play a role in the neural encoding of odorants, an homologous series of iso-intensive aldehydes differing by only one carbon from C6-C10 were observed with both behavioral and optical techniques. The behavioral technique used a rat odorant confusion matrix in which the animals were trained to differentially report (i.e., identify) each of the five different odorants. The mucosal activity patterns were optically recorded from both the septum and turbinates of 10 rats, using a voltage sensitive dye (Di-4-Anepps) and a Dalsa 128 x 128, 12 bit camera. Each odorant was randomly presented twice to each mucosal surface in a Latin Square design. Behaviorally, the animals were capable of differentially reporting the five odorants with a high degree of accuracy (> 90% correct response), even though the odorants were so chemically similar. Likewise, the mucosal activity patterns also varied in accordance with the odorant presented. In this latter regard, some activity patterns were reminiscent of the receptor zonal distribution patterns reported by a number of investigators, using cellular and molecular techniques, although within any one zone response differences were quite apparent. These optical recordings give functional support to the reported molecular observation that some odorant receptors are not uniformly distributed within a given zone.

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43 **Slide**

CLONED OLFACTORY RECEPTOR NEURONS (ORNS) EXHIBIT FUNCTIONAL RESPONSES IN VITRO.

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Olfactory epithelia from H-2Kb-tsA58 transgenic mice were isolated and grown in culture. Passage 3 cells were labeled with an anti-NCAM antibody and subjected to FACS analysis. 144 single cells were plated into 96-well plates and yielded 39 viable cell lines after expansion. Cells from one clone, 3NA12, expressed neuronal and olfactory markers including NST, NCAM, NSE, OMP, ACIII and Gαolf. Odorant stimulation of 3NA12 cells, loaded with Fura-2, caused an increase of intracellular calcium concentration in 54/1256 cells. A similar number of cells (15, 17, 12 and 16 cells, respectively) were stimulated by each odorant and six cells responded to two odorants. No cells responded to more than two odorants. These observations suggest that some level of endogenous odorant receptor expression occurs in 3NA12 cells and that the signal transduction machinery is functionally intact. In control experiments, responses to three odorant mixtures (4 odors each) were seen in 12/387 rat primary cultured ORNs, and no changes in intracellular calcium concentration were observed following odorant application in 780 olfactory bulb neurons. The low percentage of 3NA12 cells responding to odors, together with the response profiles, indicate that multiple odorant receptors may be expressed in the 3NA12 clonal cell line, though not necessarily in the same cell. This suggests that ORN progenitors may not pre-select the odorant receptor expressed by ORNs.

44 **Slide**

ODORANT STIMULATION OF CREB PHOSPHORYLATION IN A CLONAL OLFACTORY RECEPTOR NEURON CELL LINE.

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Odorant transduction in the cilia of olfactory receptor neurons (ORNs) is mediated by cAMP and results in the generation of action potentials. In addition to this immediate response, a delayed response to odorants has been also reported. This delayed response involves a secondary increase in cAMP concentration that is sufficient to activate cAMP responsive element binding protein (CREB) in ORNs both in vivo and in primary culture. Recently, we have developed 39 clonal immortal ORN cell lines from the H-2Kb-tsA58 transgenic mouse. Among these cell lines, one clone, 3NA12, has been extensively characterized. 3NA12 cells express Gαolf, adenylyl cyclase type III and olfactory marker protein, and respond to odorant stimulation with an increase in intracellular calcium concentration. This signal is believed to arise from calcium entry through the cyclic nucleotide gated cation channel. Odorant stimulation of 3NA12 cells can also activate CREB. When 3NA12 cells were exposed to citralva (10 mM) or forskolin (10 mM), CREB phosphorylation was detected from 5 to 30 minutes after the onset of odorant exposure. CREB phosphorylation was observed in cells cultured in both permissive and non-permissive conditions and was inhibited by the protein kinase inhibitor K252a (100 mM). These data indicate that CREB activation in 3NA12 cells involves cAMP activation of a protein kinase and that this pathway may be induced by odorant stimulation. These data add further support to the evidence that 3NA12 is an olfactory receptor neuron cell line.

45 **Poster**

DETERMINATION OF ODORANT SOLUBILITY IN THE OLFACTORY MUCOSA

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Odorant deposition in the nasal and olfactory mucosa is dependent on a number of factors including air stream flow rate and odorant solubility. Volume flow rate at the external naris is easily determined and numerical computer models of nasal airflow have recently been developed. For very few odorants, mucosal solubility has been determined with radioisotope techniques. To determine odorant mucosal solubility, we have applied a numerical finite element model from the fraction of odorant absorbed in the entire nasal cavity during velopharyngeal breathing (Keyhani et al., 1997) that allows the calculation of odorant solubility from the fraction of incoming odorant that passes through the nose to the nasopharynx. The fraction of odorant not absorbed by the nasal mucosa was determined by measuring the concentration of odorant passing into one nostril and, while performing a velopharyngeal closure, the concentration passing out the contralateral nostril. Odorant concentrations were measured with a photoionization detector. The fraction of odorant removed from the air stream was: ammonium hydroxide - 92%, trans-cinnemaldehyde - 93%, trans-anethole - 79%, r-carvone - 82%, napthalene - 72%, d-limonene - 58%, phenethyl alcohol - 99%, isopropanol - 60%, and acetic acid - 87% in steady airflow conditions of 10 l/min. Relative odorant solubility reflects the relative fraction of odorant removed by the nasal mucosa. Odorant solubility can be calculated through substitution into the numerical finite element model. The accuracy of our model is tested through the comparison of the output of our model and known values of odorant mucosal solubility.

46 **Poster**

PARTITIONING OF BINARY ODOR MIXTURES

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This work continues our exploration of individual differences in olfactory perception by evaluating a subject's ability to partition odor quality and quantity in binary mixtures. Four test compounds, each with a distinctive odor, were diluted and matched for intensity. They were used to make 6 binary mixtures. These mixtures and the 4 single component stimuli (plus diluent) were then evaluated by 25 undergraduate subjects (14 females) who provided two odor qualities and two intensities for each of the ten stimuli utilizing a forced choice procedure. We then asked if subjects used normative descriptors for the individual components of the stimuli and how they partitioned the intensity scores for each perceived quality. The intensities of single component stimuli across subjects confirmed that they were generally perceived as iso-intense. Under these conditions, subjects should easily identify the individual qualities in a binary mixture. We find this to be true for many people, but we also find a significant number of judgments in which the blend is described with quality reports that are different from those used for the individual components. There is an interaction between the ability to identify odor qualities in a mixture and the perceived intensity of its components. We then asked if new quality reports were more frequent in subjects who initially found the components of the mixture to be unequal in intensity. If this were so, subjects should provide quality reports dominated by the qualities of the stronger odor. In most cases, new qualities were more frequent in subjects who perceived similar intensities. The frequency of new quality labels for mixtures is also influenced by affect. Subjects provide fewer new descriptors with mixtures containing unpleasant odors. Mixture suppression was pronounced, but unequally applied to the two components. These results suggest additional individual differences in odor perception among subjects.

47 Poster

RETRONASAL AND ORTHONASAL IDENTIFICATIONS OF VAPOR-PHASE FOOD-GRADE LIQUID EXTRACTS OF PLANT MATERIALS

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A total of forty-four unscreened male and female subjects were tested in three experiments. Separate odorant presentation containers (Pierce and Halpern, Chemical Senses, 21, 529-543, 1996) were used for retronasal and orthonasal presentations and for each odorant, and discarded after each subject. Orthonasal sniffing was not permitted; modified retronasal breathing was not taught. FIRST, subjects provided identifications, and were corrected if wrong. SECOND, presentations were made to the nares not used for the first step, and no corrections were made. THIRD, presentations were made to the nares used for the first step, and no corrections were made. A printed list of veridical odorants names, plus distracting names in some experiments, was provided. In some experiments, the three steps were repeated, with the nares sequence reversed from the initial sequence. RESULTS: With undiluted odorants, uncorrected orthonasal identifications were veridical on 91% (+/- 3%) of trials (median +/- SIR); retronasal, 86% (+/- 4%). Orthonasal percentages ranged from 100% for wintergreen to 63% for lemon; retronasal, 100% for banana to 64% for lemon. Confusion Matrices (H. N. Wright, Arch. Otolaryng. 113, 163, 1987) showed 28% orthonasal confusion between lemon and orange; retronasal, 14% to 20%. Substantial individual differences occurred. With 1:2 diluted odorants and orthonasal learning, median uncorrected retronasal identifications were 88% (+/- 25%) correct; orthonasal, 100% (+/- 0%), and retronasal % correct significantly different from orthonasal, $p = 0.005$. All orthonasal identifications = median of 100% except orange, 75%; lemon, 50%. Retronasal, orange 75%, lemon, coffee, (canola) oil, 50%. Individual nares differences ranged from zero [100% (+/- 0%) retronasal and orthonasal] through 50% (+/- 50%) retronasal and 100% (+/- 31%) orthonasal to 0% (+/- 6%) correct retronasal and 100% (+/- 25%) orthonasal. After subsequent retronasal learning, diluted odorant difference not significant.

48 Poster

ODOR IDENTIFICATION: HOW TO TELL IF SUBJECTS ARE RIGHT WITHOUT LOOKING AT THEIR RESPONSES

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According to our recent studies on scores of subjects, when people seek to identify odors they tend to emit correct labels more consistently (i.e., apply the same label to a given odor in separate sessions), with greater confidence, and more quickly than they emit incorrect labels. An experimenter can therefore judge the correctness of a response to an extent without looking at it. However, the three dependent variables, alone or in combination, have failed to provide perfect resolution between nominally correct and incorrect labels. The current study sought to increase resolution with two methodological changes: 1) Previously, only reasonably specific answers counted as correct. A subject might, for example, apply labels such as "fruit" or "citrus" to orange essence quickly and with high confidence, even though these were by definition incorrect. The instructions for the current study emphasized the need for specific answers. 2) Previously, subjects tended to emit some labels multiple times during a session, even though they received each stimulus only once. Consistent application of some labels could have been due to chance rather than stable perception/retrieval. The instructions for the current study cautioned subjects against applying a label twice within a session unless very certain of the second application. With these methodological changes in place, the ability of consistency, confidence, and latency to resolve between correct and incorrect responses improved considerably. Resolution still fell short of perfection, and accordingly leaves some room for improvement. However, a combination of consistency, confidence, and latency yields a reasonably precise, objective index of the ability to identify odors.

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49 Poster

EFFECTS OF MASKING ON ODOR IDENTIFICATION

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An Olfactory Confusion Matrix (OCM) was used to determine effects of a masking odor on identification of two different sets of odorants. In experiment 1, 12 subjects identified eight familiar household items: baby powder, chocolate, cinnamon, coffee, mothballs, peanut butter, Ivory® soap, and Vicks®. In experiment 2, 11 subjects identified eight pure chemicals with less familiar odors: 50% (v/v) eugenol ('cloves'), 1% butyric acid ('cheese'), 50% anethole ('licorice'), 2% amyl acetate ('banana'), 50% l-carvone ('spearmint'), 25% d-carvone ('rye' bread'), 50% phenyl ethyl alcohol ('rose'), and 5% citral ('lemon'). The odorants were presented randomly ten times each with and without a masking odor: peanut butter in experiment 1 and butyric acid in experiment 2. Subjects correctly identified unmasked household items 95.5±1.0% of the time, but identified peanut-butter masked odorants less frequently: 89.7±1.9% ($p<0.006$). Consistency of identification: T_8, bits of information transferred, was lower in the masked set (2.6±0.07 bits) compared to the unmasked (2.8±0.04 bits)($p<0.013$). The peanut-butter masking odor reduced average pairwise discriminability (T_2) of baby powder ($p<0.001$), chocolate ($p<0.012$), and mothballs ($p<0.010$) vs. other odorants. Neither overall percent correct (88.4±2.0% for unmasked, 85.6±2.2% for masked) nor T_8 (2.6±0.08 bits vs. 2.5±0.08 bits) was significantly affected by butyric-acid masking of the pure odors. Yet, average T_2 for amyl acetate ($p<0.04$) and butyric acid ($p<0.011$) were reduced with masking. In spite of training trials given all subjects, performance for the set of pure chemicals did not reach the level attained for the set of common items. We conclude that the OCM technique can measure effects of a masking odor on olfactory function. The degree of masking depends on the masking odor and the test array.

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50 Poster

AMELIORATING SWINE SLURRY ODORS: AN ANALYTICAL AND SENSORY APPROACH

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Odors from some agricultural practices can create conflicts at the urban-rural interface. Conflicts may displace these operations or force a reduction in production to limit odors. Either of these solutions can impact negatively upon the economic interests of the agricultural community. To help minimize potential odor-mediated conflicts, we have been exploring mechanisms to reduce or eliminate malodor in swine farming operations. These have received considerable focus for producing malodors in the local community.

The effectiveness of five treatments (absorbents, deodorizers, and cross adapting compounds) in reducing the stench of swine slurry was evaluated by a sensory panel, as well as analytically by gas chromatography (GC), mass spectrometry (MS), and gas chromatography/olfactometry (GC/O). One percent (by weight) powdered activated carbon combined with one percent (by weight) bismuth citrate in conjunction with either 0.25% of 3-methyl-2-pentenoic or 3-methyl-2-octenoic acid ethyl esters were found to decrease headspace volatile concentrations the greatest, and the subsequent odor was deemed least unpleasant by a sensory panel. Volatile sulfur components (hydrogen sulfide, methyl mercaptan, and dimethyl sufide) as well as the volatile organic compounds: phenol, cresol, p-ethyl phenol, indole, and skatole were among the most malodorous constituents in the swine slurry odor by GC/O experiments. Odor descriptors used to describe the constituents as they eluted from the chromatograph were egg-like, fecal, ham, barnyard, earthy, sour hay, and animal. Our results suggest several constituents which may be used as either feed supplements for swine or post-hoc additives to the slurry pit to diminish the offensive odor.

51 Poster

FEEDING OF SWINE TO AMELIORATE ODORS: AN ANALYTICAL AND SENSORY APPROACH

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Odors from some agricultural practices can create conflicts at the urban-rural interface which may displace these operations or force a reduction in production to limit odors. Either of these impacts negatively upon the economic interests of the agricultural community, wherever that may be. To minimize this discord, we have been exploring mechanisms to reduce or eliminate malodors in swine farming and management. We have pursued analytical and organoleptic approaches. In another presentation the focus in on direct treatment of swine slurry (SS). In this presentation, the focus is on manipulations of feed and SS.

Human volunteers can reliably rate odor intensity using the Labeled Magnitude Scale, a psychophysical metric that relies upon an individual's life-time experiences with smell. Resulting ratings from Barely Perceptible to Strongest Imaginable are converted to numerical values for analysis. Odor pleasantness can be quantified by using a categorical rating-scale, ranging from -11 = Extremely Unpleasant, through 0 = Neutral, to +11 = Extremely Pleasant. Using these tools we obtained multiple ratings over time of SS, treated with (or without) powdered activated charcoal, from pigs maintained on different diets, viz., feed with copper chlorophyllin (CC), CC + bismuth, bismuth alone, $CuSO_4$, or feed alone. We also performed analytical evaluations of SS, using the same samples that were subjected to odor evaluation by a panel of 16 judges.

Results suggested that the dietary manipulations were not successful in reducing malodors, at least at the high concentrations of neat SS; however, treatment of SS with activated charcoal significantly reduced the perceived intensity of the samples, its perceived unpleasantness, and analytical measures of malodorous compounds. Additional studies are underway to determine whether dilution, e.g., down-wind sampling, reveals differences among the groups of pigs fed different diets. Supported by PA Dept of Agriculture and NIH (DC00298).

52 Poster

THE UNPLEASANTNESS OF MIXED AND UNMIXED MALODORS: ASSESSMENT BY THREE METHODS

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We have recently initiated a program of research directed toward the development of a clinical test of olfaction. Our approach is based on the measurement of sniffing behavior in the presence of a malodor. One of the problems we face is the selection of a malodor that is uniformly effective in reducing the size of sniffs for anyone who has a normal sense of smell. Research by Laing et al. (1994) indicates that mixtures of malodorants are perceived as more unpleasant than individual components. In addition, using a mixture reduces potential problems associated with specific anosmia, as well as individual differences in hedonic responses to particular odorants. The current study represents an extension of Laing's work using different methods and odorants. Single odorants and their mixtures were evaluated using the Label Magnitude Scale (LMS), direct comparisons of odor pairs, and several measures of sniff magnitude. Whereas Laing mixed odorants to create ecologically valid malodors (e.g., sewer gas), our approach in this pilot study was to begin work on creating the worst possible smell. Participants were students from the University of Cincinnati introductory psychology pool. Single odorants and their mixtures were rated using the LMS that was adapted to assess the unpleasantness of an odor on a scale from barely detectable to most unpleasant imaginable. Air pressure changes over time were measured to assess sniffing behavior. In addition, participants selected the more unpleasant of two smells using a paired comparison procedure. All three testing methods showed that the stimulus composed of the most malodorants (a four component mixture) was most unpleasant. The next step is to conduct additional tests with a wide variety of malodorants to optimize the unpleasantness of the test mixture.

53 Poster

THE INFLUENCE OF BELIEF VERSUS CONTENT ON THE PERCEPTION OF NATURAL AND SYNTHETIC ODORS

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The influence of beliefs about the "naturalness" of odor composition, in contrast to actual odorant composition, on evaluations of olfactory perception was examined. Forty subjects were tested in two experimental sessions with a series of eight familiar odors presented in either their natural or synthetic form. Half of the odors used were pleasant and half unpleasant. At Session 1, subjects were asked to guess each odorant's composition (natural or synthetic) and then to rate the odor on various scales. One week later, at Session 2, subjects were presented with the same odors to rate, but this time were told either that all the odors were "natural essences" or that they were all "synthetic-chemicals. " At both sessions, subjects received four of the odors in natural form and four in synthetic form, however, this was not revealed to them until the experiment was over. Analyses of the responses given at Session 1 showed no differences in the ratings made on any scale between natural and synthetic odors. However, subjects rated odors that they believed were natural as significantly more pleasant, safe and familiar than odors they believed were synthetic. Positive odors were also rated as more pleasant, safe, familiar, stronger and calming than negative odors. In Session 2, odors were rated as safer when subjects were told that the odors were natural and also when the odors smelled pleasant. Rating changes between Session 1 and session 2 showed that when subjects were told the odors were natural, pleasantness ratings for positive odors increased dramatically. These findings demonstrate that in blind testing responses to natural and synthetic versions of the same odorants are indistinguishable. However, beliefs about odorant composition strongly influences perception, with perceived "naturalness" enhancing hedonic quality along a variety of dimensions.

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54 Poster

RECOLLECTIVE EXPERIENCE OF ODORS AND WORDS: EFFECTS OF LEVEL OF PROCESSING, RETENTION INTERVAL, AND ODOR IDENTIFIABILITY

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Two experiments investigated how episodic recognition of odors and words varied as a function of level of processing (LOP) and retention interval (RI). Two aspects of memory was measured: performance (Aâ??), through the yes/no procedure, and experience, through the remember/know procedure (e.g., Gardiner & Java, 1993). Words, in comparison to odors, were better recognized and were more dominated by explicit recollection of the stimulus encounter (as indicated by the number of "remember" responses). Word memory was also more sensitive to experimental manipulations. For instance, word recognition performance was significantly enhanced by deeper processing at study, whereas odor recognition was not. Word recognition, more than odor recognition, gained in number of remember responses as a function of shorter RI. However, these differences between word and odor memory are interpreted to be of a quantitative rather than qualitative nature. To investigate the hypothesis that the discrepancies between word and odor memory was due to the fact that many odors are hard to identify, a third experiment was performed. Odor stimuli were divided in two sets based on identification scores assessed in Experiment 1. LOP at study was also varied. Highly identifiable odors were associated with higher levels of recognition performance and more remember responses. However, odors of high as well as low identifiability were insensitive to LOP, both in terms of recognition performance and experience.

55 Poster

VERBAL-COGNITIVE STRATEGY CAN INFLUENCE ODOR IDENTIFICATION.

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Wine assessment commonly involves sniffing the volatiles from a wine sample then mentally searching for an appropriate descriptor. This is often a difficult task in terms of speed and accuracy. Can the task of finding the descriptor be made more accurate by use of various cognitive strategies? We asked 650 people to perform one of two strategies: one required the subject to read a descriptor then search for an odor to match it (from a set of 5 odor options in plastic squeeze bottles); the other required the subject to sniff a single odour and find the appropriate descriptor from a list of five descriptor options. The task of finding the word to match the sniffed odor was significantly more difficult than the task of being given a word and asked to find the matching odor. The odors were not uniformly easy to identify: lemon was most accurately identified, followed by mint, rose, burnt, then almond. Age and gender had insubstantial effects on results. People whose home language was not English made more errors on all variations of the task. Bottles which had a color congruent with the odor (e.g rose = pink, lemon = yellow) significantly decreased the identification errors compared with white and incongruently colored bottles, and had the greatest effect on the differences in errors between the two tasks. This suggests that correct color information facilitates the more effective cognitive strategy. Sniffing an odor appears to create an attentional block to the retrieval of verbal information or the usefulness of odor-related sensory information such as color-associations. Wine judgement and appreciation should be improved by adoption of and training in the better of these two strategies.

56 Poster

MOOD, PERSONALITY, AND ODOR PERCEPTION

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Both personal experience and existing research suggest that scents and odors can modulate one's mood state. What has been less well-examined is whether personality and current mood states can influence how odors are perceived. The purpose of this study is to examine the effect of extraverted and neurotic personality and happy (H), sad (S), angry (A), and neutral (N) moods on people's responses to and evaluations of pleasant, unpleasant, and neutral odors. Psychological research reveals that both personality and specific mood states can be associated with distinct perceptual and cognitive patterns that "bias" the way people attend to external stimuli. In this study, we induced H, S, A, and N moods using short video segments, and examined subjects' response latencies to an odor following each segment, their videotaped facial expressions, and their perceptions of odor pleasantness and intensity as a function of mood states and personality dimensions. All subjects watched a total of 12 movie segments presented in 4 blocks, each segment was shortly followed by an odor. The movie and odor presentations were counterbalanced within each block such that each odor had an equal chance of being paired with a happy, sad, angry or neutral movie. The movie segments were chosen based on both established studies and on prior evaluations by a panel of judges. An examination of self-reported mood showed that the mood induction was successful. Overall, the results suggest that the process of odor perception is guided both by features of the stimulus and the characteristics of the perceiver.

57 Poster

ODOR CHARACTERISTICS OF BREASTFEEDING CHEMOSIGNALS

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Chemosignals from breastfeeding mothers and their infants alter the length of recipient women's menstrual cycles (Spencer et al., AChemS 2000 abstract). All 47 women in this double-blind, randomized, between- and within- subject designed study were exposed to the carrier control compounds for one month (cotton pads, moistened with sodium phosphate buffer solution to mimic the substrate of sweat and breastmilk). In the next two experimental cycles, approximately half of the women continued to receive the carrier control, while the other half received axillary and breast pads collected from 26 lactating women, hereafter referred to as breastfeeding chemosignals. These pads contained mothers' milk, maternal body odors and most likely infant secretions (e.g. saliva) since mothers nursed during collection periods. Recipient women wiped the breastfeeding or carrier control pads directly under the nose up to four times daily. During biweekly laboratory visits throughout the three month exposure period, the women rated the intensity and hedonic quality of the pads and recorded what they thought the odors were and their associations, if any, to the odors. We also measured the onset of the preovulatory LH surge of each recipient woman in order to demarcate the three major phases of the menstrual cycle. From these data, we can determine the recipient women's perceptions of the breastfeeding chemosignals throughout the exposure periods and cycle phases. Detailed analyses are forthcoming.

58 Poster

EFFECTS OF SOME HUMAN ASSOCIATED ODORS ON THE BEHAVIOUR IN THE INITIAL ENCOUNTER

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The study tests if estratetraenol(EST) and androstadienol (AND) have an influence on "flirtation" behaviour (part of human courtship behaviour;e.g. defined by Moore (1985) and Tramitz (1992). In our study 1 man and 1 women were invited for a visual discrimination test. They were prepared separatly. One of them was sitting on a chair behind a curtain. The other was led in.First it was not possible for them to see each other because they were separated by a curtain. In the treatment group EST was applied to the cheek of the man and AND was applied to the women (explained by a cover story). The solvent was applied to the cheeks in the control. The experimenters removed the parting curtain and left the room. With the removal the data collection started and the subjects were video taped by a hidden camera for 10 min while waiting for the test. After this the test was conducted. A questionnaire was administered. It included hidden questions about how they assess the other and what they would like to do. Subjects were informed about being filmed and the aim of the study. Data was analysed when the subjects gave their permisssion. Otherwise the data was deleted. The tapes were analysed concerning body positions, movements, flirtation signals and speech. 120 men and 120 women took part in the study. Men and women in the treatment group e.g. would take sig. more initiative to get in contact with each other and feel hurt if they were rejected than undercontrol. Both sexes e.g. showed sig. more coy smiles in the treatment group; but e.g. head and trunk were more often turned away than under cotnrol.

59 Poster

EFFECTS OF THE PUTATIVE PHEROMONE 4,16-ANDROSTADIEN-3-ONE ON PSYCHOLOGICAL AND PSYCHOPHYSIOLOGICAL VARIABLES: WEAK EVIDENCE

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A chemical emitted from one animal that exerts a behavioral or physiological response in another animal of the same species has been termed a pheromone. Recently, some reports (Monti-Bloch et al., 1998; Jacob & McClintock, 1999) indicate that 4,16-androstadien-3-one (androstadienone) is a putative human pheromone. Data are, however, scarce. Therefore, an experiment testing the effects of androstadienone on psychological and pshychophysiological variables was performed. Twenty clinically normal female human volunteers participated in a double blind, repeated measures experiment that was counterbalanced for treatment order. All of the participants used barrier contraceptives and none of them were to their knowledge pregnant. All participants were exposed either to a solution containing one micromolar androstadienone dissolved in mineral oil (test stimulus) or to pure mineral oil (control stimulus). The stimuli were presented to the participants in glass jars. Mood tests, measures of heart rate (HR), respiratory rate (RR), and skin conductance (SC) were administrated during 5 min before and 8 min after the stimulus exposure. A discrimination test after the experiment revealed that participants could not discriminate between the test and control stimuli. The results were that androstadienone did not significantly affect the general level of HR, RR, SC, or mood. However, the recovery of RR after the exposition was significantly faster for androstadienone than for the control stimulus.

60 Poster

EARLY LEARNING ABOUT THE SENSORY PROPERTIES OF ALCOHOL

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Previous research in our laboratory revealed that during the first year of life, infants who had more exposure to alcohol, as inferred from questionnaires about parental alcoholism and alcohol intake, behaved differently in the presence of an ethanol-scented toy when compared with less exposed infants. The present study focused on 4- to 6-year-old children (n = 150) to determine whether their hedonic response to the odor of alcohol was related to the drinking 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 preferences for a variety of odors, one of which was alcohol. The study revealed that the preference for the smell of alcohol is related to parental drinking habits. That is, children of parents who drink alcohol for escape reasons were significantly more likely to dislike the odor of ethanol when compared to similarly aged children whose parents did not drink to escape. This differential response was specific to the odor of alcohol. These findings suggest that some early learning about alcohol is based on sensory experiences and anchors it to children's experiences at home and the emotional context in which alcohol is experienced.

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61 Poster

SENSORY CONSEQUENCES OF OCCUPATIONAL EXPOSURE TO ISOPROPYL ALCOHOL

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Workplace exposure limits for many volatile chemicals are based on the lowest concentration that elicits sensory irritation. To determine these limits, many studies have asked subjects to rate perceived irritation at different exposure concentrations. However, asking a subject to rate irritation and ignore odor may bias the ratings. In this study we compared an objective method (nasal lateralization) with scalar ratings to determine the irritation threshold for the industrial solvent isopropyl alcohol (IPA). Additionally, we explored whether repetitive exposure to IPA decreased perceived irritation from IPA, by comparing thresholds and perceived odor and irritation among a group of workers with occupational exposure to IPA and a control group.

We obtained odor detection thresholds for IPA, a control irritant (1-butanol), and a control odorant (Phenylethyl alcohol) from 25 phlebotomists, with daily exposure to IPA, and 25 matched controls. The threshold for nasal sensory irritation to IPA (and 1-butanol) was determined using the lateralization method. This method is based on the principle that by stimulating the trigeminal nerve peripherally in one nostril, the sensation can be lateralized to that nostril.

The average lateralization threshold for IPA was significantly higher for the exposed group (8445 ppm) than for the controls (5676 ppm), while lateralization thresholds for 1-butanol were comparable among groups. No group differences were found for odor detection thresholds for any chemical. Subjective ratings of irritation for three different concentrations of IPA that were based on each individual subject's odor and lateralization thresholds did not reveal any between-group differences.

We concluded that: 1) the subjective intensity ratings were actually comparable to objective measures when standardized to the individual subject's thresholds, and 2) occupational exposure to IPA was associated with elevated irritation thresholds for IPA, but not 1-butanol, suggesting specific adaptation of the trigeminal nerve in the nose to IPA.

62 Poster

UNILATERAL ANESTHESIA OF THE CHORDA TYMPANI NERVE SUGGESTS TASTE MAY LOCALIZE RETRONASAL OLFACTION

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Retronasal olfaction is perceptually localized to the mouth. Oral somatosensory stimulation (eg, palpating, chewing, swallowing) appears to play a role in this localization. We propose that taste stimulation also plays a role. Clinical observations of Bull (1965) suggested a role of taste in retronasal olfactory perception; individuals with surgical damage to the chorda tympani nerve (taste, anterior tongue) reported alterations in food flavor that reflected taste but also retonasal olfaction (eg, inability to distinguish tea from coffee). In our study, 20 healthy young adults undergoing unilateral anesthesia of the chorda tympani provided an opportunity to examine how temporary manipulation of taste may affect retronasal olfaction. Halpern and Nelson (1965) demonstrated inhibition between the chorda tympani and glossopharyngeal (taste, posterior tongue) nerves. Lehman et al (1995) and Yanagisawa et al (1997) confirmed this in humans; taste was intensified at the contralateral glossopharyngeal nerve when the chorda tympani nerve was temporarily blocked. In the present experiment, subjects sampled blueberry yogurt after unilateral chorda tympani anesthesia and confirmed loss of taste sensation. Subjects were asked if "blueberry flavor, not sweet, not tart, appears to be coming from any particular part of the mouth." Fourteen subjects localized the blueberry flavor to the posterior, unanesthetized side of the tongue (sign test, p= .005). Ten of the subjects participated in a second session in which the other chorda tympani was anesthetized; eight again localized blueberry flavor to the posterior of the unanesthetized side of the tongue (sign test, p = .01). In both cases, blueberry flavor was diminished on the area of the tongue where taste was absent. These findings suggest that taste plays a role in the perceptual localization of retronasal olfaction.

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63 Poster

BUBBLE, BUBBLE: PERCEPTION OF A CARBONATED BEVERAGE ACROSS THE LIFESPAN

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The goal of this work was to understand perception of carbonated beverages across genders and across the life-span and to understand how age-related olfactory change influences the appreciation of carbonated beverages. We have found age-differences in intensity perception of carbonation between teens and other age groups. The teens had steeper psychophysical functions than other age-groups: Teens rated the low concentrations lower and the high concentrations higher than did the adults. It is important to emphasize that we found no difference between young adults and elderly adults in oral perception of this irritant. Teens liked the beverages better than did members of other age groups and this is consistent with published marketing trends. Overall, low concentrations of carbonation were liked better than high levels and males liked highly carbonated beverages better than females did. We documented some relationship between liking for the beverages and olfactory sensitivity: Individuals with better senses of smell liked the beverages better. It is also known that olfactory sensitivity of older individuals is lower, on average, than it is in young adults. This may contribute, in part to the age differences in use of and liking for carbonated beverages. However, this effect, in our study, was not large and cannot, in itself, account for age differences in preference. The major conclusion is that lower levels of carbonation may be appropriate for some segments of the population. It is unknown at this time whether the age and gender differences have a physiological mechanism, whether they are related to differences in individual experience, or both.

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64 Poster

COLOR AFFECTS PERCEIVED FLAVOR INTENSITY

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Color increases perceived odor intensity when solutions are smelled (Zellner & Kautz, 1990; Zellner & Whitten, 1999). The present study suggests that this might not be the case when the solution is ingested rather than just smelled.

Thirty-two subjects drank and rated the intensity of the mint flavor of four different solutions twice. Two of the four solutions were spring water, one with green food coloring and one colorless. The other two solutions were equally concentrated mixtures of mint syrup in spring water, one with green food coloring and one colorless. The four solutions were presented twice in random sequences. Subjects tasted and rated the "mintiness" of a solution once every 30 seconds using a 100-point scale (0 labeled "no flavor", 50 labeled "moderate", 100 labeled "the most intense flavor imaginable"). Subjects rinsed their mouths with spring water between samples.

The first set of ratings were considered practice trials. A significant difference in mint intensity ratings was found among the four solutions (Friedman Chi-square(3) = 86.35, p<.001). Wilcoxon tests showed that solutions containing mint syrup were rated significantly more minty than those without mint syrup (all p<.001). Green-colored spring water was rated significantly more minty than colorless spring water (Z = 2.41; p = .016). However, the green-colored mint solution was rated less minty than the equally concentrated colorless mint solution (Z = 2.26, p = .024).

If the green-colored mint solution smells stronger than the colorless leading the subject to expect a stronger flavored solution than they get when it is in their mouth the decrease in its mintiness rating when tasted could be the result of contrast between anticipated and experienced flavor.

65 Poster

INTERACTION OF FAT WITH A RANGE OF TASTANTS AND TRIGEMINAL STIMULANTS

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Fats contribute to the sensory experience of food. Reducing fat changes the food's textural, mouthfeel and volatile release characteristics, decreasing its palatability. It was thought that fats had no input in the gustatory system, because deodorised fat is flavourless. However, Gilbertson et al. (1997) demonstrated that isolated rat taste cells respond to cis-polyunsaturated fatty acids via inhibition of Kdr channels, suggesting a receptor mechanism for transduction of fat "taste".

This study investigated whether mixture interactions observed between different taste qualities (e.g. suppression of bitter by sweet) are also evident for interactions between fat and tastants, and between fat and trigeminal stimulants. Stimuli were model emulsions of similar viscosity, varying in the level of deodorised safflower oil and xanthan gum, with tastants incorporated into the emulsions. Using line scales, fourteen trained subjects rated the taste intensities of 75mM NaCl, 100mM Sucrose, 100mM Citric Acid, 92mM Quinine HCl, 2.16mM Caffeine and 30mM MSG at 0, 10 and 20% safflower oil. In addition, the burn intensities of pepper (1.67g/L) and ginger (5.0g/L) were evaluated at 0, 12.5 and 25% safflower oil.

ANOVA results showed that while there were no effects of the level of oil on the perception of primary taste qualities, the savoury taste intensity of MSG increased significantly with the level of oil (p = 0.038). Conversely, increasing the level of oil significantly decreased the burn sensation of pepper (p = 0.006) and ginger (p = 0.00008). These results indicate that the sensory effects of dietary fat go beyond its textural and olfactory effects, to also influence savoury taste and trigeminal perception. Fats may interfere with the receptors for glutamate, piperine and gingerol in order to convey information from the periphery to the brain or they may modify those processes dealing with such information.

66 Poster

INFLUENCE OF EVERYDAY ACTIVITIES ON THE VIGILANCE STATE IN HUMANS

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It is generally known that vigilance in humans changes over time. External environmental influences and activities are thought to be responsible for these changes. Subjectively assessed vigilance state can be correlated to EEG activity and event-related potentials (Koelega et al., 1992).

The aim of this study was to investigate changes in vigilance during everyday activities by using electrophysiological parameters such as VEP and EEG.

The vigilance state of 30 healthy young volunteers (15 male/15 female) was determined prior to and after an everyday activity lasting 10 minutes. Several different activities were tested: drinking regular coffee or caffeine-free coffee, watching a music video clip, doing mental calculations, smelling menthol, jasmine, or lavender, and smoking a cigarette. A non-activity condition, i.e., without any instructions to or treatment of the subjects, was included. Vigilance state was determined by measuring pattern reversal evoked potentials (PREP) and background activity of the EEG. PREPS were recorded twice - 3 minutes prior to and after the activity. Pre-post differences in latencies and amplitudes were evaluated. FFT analysis of the background EEG was performed.

A comparison of PREP-pre and -post differences revealed a decrease in latency P1 for smoking (2.26 ms), mental calculation (0.7 ms), drinking regular coffee (0.5 ms). These activities were statistically significantly different from the non-activity condition. The 3 odorants did not differ significantly from the non-activity condition. Drinking decaffeinated coffee or watching music video clips showed no distinct pre-post difference.

Changes of the background activity of EEG confirm the results of PREPs for most of the activities tested. It was demonstrated that the PREPs and background activity are useful tools with which to measure the influence of everyday activities on vigilance. Smoking a cigarette, drinking coffee, and doing mental calculations apparently increase vigilance. Smelling of the odorants tested did not affect vigilance in this study.

(Sponsored by Philip Morris U.S.A.)

67 Poster

PRE- AND POST-NATAL EXPOSURE TO THE FLAVOR OF CARROTS AFFECTS THE INFANTS' ACCEPTANCE OF CARROT-FLAVORED CEREAL

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Does experience with a flavor in amniotic fluid or mothers' milk modify the infants' acceptance of similarly flavored foods at weaning? To investigate this question, we randomly formed three groups of pregnant women (n = 45) who planned on breastfeeding their infants. The women consumed either 300ml of carrot juice or water for 4 days per week for three consecutive weeks during the last trimester of pregnancy and then again during the first two months of lactation. The mothers in Group 1 drank carrot juice during pregnancy and water during lactation; mothers in Group 2 drank water during pregnancy and carrot juice during lactation whereas those in Group 3 drank water during both pregnancy and lactation. Approximately 4 weeks after the mothers began complementing their infants' diet with cereal, the infants, who were, on average, 5.5±0.1 months, were videotaped as they fed, in counter-balanced order, cereal prepared with water on one testing day and cereal prepared with carrot juice on the other. Infants fed at their customary pace until they refused the cereal three consecutive times. Immediately after each feeding session, the mothers rated their infants' enjoyment of the food on a 9-point scale. The results demonstrated that the infants who had exposure to the flavor of carrots in either amniotic fluid or mothers' milk consumed significantly more of the carrot-flavored cereal and were perceived by their mothers as enjoying the carrot-flavored cereal more when compared to infants without such exposure. These findings are the first experimental evidence to demonstrate that exposure to a flavor, either pre- or post-natally, influences the human infants' acceptance and enjoyment of similarly flavored foods.

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68 Poster

DEVELOPMENT OF BRIEF METHODS TO CLASSIFY INDIVIDUALS BY PROP TASTER STATUS.

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There are genetic differences among individuals in the perceived bitterness of PROP, that are related to the perception of other bitter tastes, sweet taste, the burn of capsaicin and the mouthfeel of fats. Bartoshuk et al. (1994) devised a now popular method for PROP classification in which subjects judge the intensity of 5 solutions each of PROP and NaCl. Classification is determined by visually comparing the psychophysical function for PROP to that of NaCl for each subject. Because this procedure is time consuming, requires tasting many samples and is sensitive to experimenter error in classification, brief and reliable methods are needed. This study describes 2 brief classification methods based on the Bartoshuk procedure. 89 adult employees of the Colgate-Palmolive Co. participated in the study. Subjects rated the perceived intensity of solutions of PROP (0.032, 0.32, and 3.2 mM) and NaCl (0.01, 0.1, 1.0 M) (3-solution test) and solutions of 0.32 mM PROP and 1.0 M NaCl (1-solution test) using the Labeled Magnitude Scale (LMS). Each test was completed twice and the mean of the two observations was calculated. Subjects were classified as nontasters (n = 22) μεδιυμ ταστερσ (ν = 52) or supertasters (n = 16) on the 3-solution test. Group functions were not statistically different from those obtained in a previous study using the standard 5-solution method (Tepper & Nurse, 1997). Taster status in the 1-solution test was determined using numerical cutoffs obtained by constructing 95% confidence intervals around the group means for PROP. A rating of 51 (corresponding to "very strong" on the LMS) defined the supertasters and a rating of £ 15.5 (approximately "moderate" on the LMS) defined the nontasters. 85% of subjects were similarly classified by the two methods. These data suggest that 3- and 1-solution methods can reliably classify subjects by PROP taste sensitivity and could be valuable in population-based studies.

69 Poster

TRANSMISSION OF OLFACTORY INFORMATION FROM THE EPITHELIUM TO THE BULB OCCURS VIA GLUTAMATE RELEASE IN ZEBRAFISH.

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Previously, we demonstrated widespread glutamatergic activation of zebrafish olfactory bulb neurons using agonists to ionotropic glutamate receptors (IGR), which suggested its involvement in olfactory transmission (Edwards and Michel, Chemical Senses 24(5):534, 1999). In the current investigation, we confirm that glutamate mediates transmission of olfactory information to and within the olfactory bulb. Since amino acids and bile salts activate distinct regions of the olfactory bulb in zebrafish (Friedrich and Korsching, J. Neurosci. 18(23):9977, 1998), we used L-glutamine (Gln;100μM) and taurocholic acid (TCA;10μM) as odorants in our experiments. One of these odors or an artificial fresh water control was applied to the nose, while the exposed brain of an anaesthetized zebrafish was simultaneously perfused with artificial cerebrospinal fluid containing agmatine (AGB;5μM), a nonspecific cation channel permeant probe, or AGB + IGR antagonists (100μM APV and 50mM CNQX). Under control conditions, AGB labeling in mitral and granule cells is low and was blocked by IGR antagonists, indicating the presence of basal activity. Odor stimulation with either Gln or TCA significantly increased the intensity and number of AGB labeled mitral and granule cells. IGR antagonists reduced this labeling to very low levels. Thus, we conclude that odor-induced activation of mitral cells is mediated predominantly by presynaptic glutamate release at olfactory receptor neuron-mitral cell synapses. Not surprisingly, odor stimulated AGB labeling of the bulb was not uniform. Gln stimulated labeling was restricted to lateral glomeruli, mitral and granule cells. TCA stimulated labeling was restricted to medial glomeruli, mitral and granule cells. The observed labeling of granule cells following odor stimulation is presumably driven by glutamate release from activated mitral cells. Collectively, these findings indicate that both medial and lateral pathways in the fish olfactory bulb transmit odor information via glutamatergic synapses.

70 Poster

THE SPATIAL ORGANIZATION OF OLFACTORY RECEPTOR AXONS AND OF SEROTONERGIC (5HT) FIBERS IN THE GLOMERULAR AND MITRAL CELL LAYER OF THE OLFACTORY BULB IN THE LARVAL LAMPREY.

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In the lamprey, the primary olfactory pathway contains 5HT immunoreactive (IR) fibers that originate from the cell bodies beneath the olfactory and nonsensory epithelium, and extend along the olfactory nerve (Zielinski et al., J. Comp. Neurol., In press). Some 5HT fibers terminate in the medial portion of the olfactory bulb, and some course along the dorsal and medial surfaces of the olfactory bulb. In this study, we investigated the spatial organization of olfactory glomeruli using histochemical staining with Griffonia simplicifolia-1 (GS-1) lectin. The glomerular units lacked glial borders, and individual glomerular units were not well defined. A pattern of 7 glomerular groupings was observed in each lamprey: a dorsal cluster, an anterior plexus, a lateral chain, a plexus of the lateral chain, a medio-anterior glomerulus, a medial elongated glomerulus, and a small grouping of ventral glomeruli. In the ventral region, GS-1 fibers extended through the olfactory bulb, to the diencephalon. The 5HT-IR fibers extended along the edges of the dorsal cluster, and passed through the space separating the anterior group from the lateral chain. 5HT-IR fibers flanked the edges of posterior glomerular units of the lateral chain. The 5HT-IR fibers that entered the medial region of the olfactory bulb from the olfactory nerve terminated adjacent to the medio anterior glomerulus. 5HT fibers were rarely observed in the anterior and ventral subregions. This concentration of 5HT fibers in the dorsal, lateral and medial subregions of the glomerular and mitral cell layer, suggests modulation of specific synaptic events that occur in these subregions of the lamprey olfactory bulb.

71 Poster

IMMUNOLOCALIZATION OF OLFACTORY CYCLIC NUCLEOTIDE GATED CHANNEL A -SUBUNIT (OCNC1) IN MOUSE OLFACTORY BULB AND CORTEX

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Previously, we and others showed that expression of cyclic nucleotide-gated (CNG) channels is not restricted to sensory neurons, but that transcripts of CNG channel mRNA can be found in many neurons in CNS. To begin to analyze the functions of CNG channels in central neurons, we are investigating the localization of CNG channel protein at specific sites in the CNS. Here, we focused on higher centers of the olfactory system. Using an affinity-purified polyclonal antibody that recognizes the olfactory CNG channel α-subunit (Bradley et al., 1997), we found that, in the main olfactory bulb, immunoreactivity was intense in axons of the olfactory nerve layer and in glomeruli. A small subpopulation of juxtaglomerular somata were labeled. Immunoreactive neurites could occasionally be seen connecting glomeruli. In the external plexiform layer, tufted cells and their primary dendrites were weakly labeled; we also observed weakly labeled mitral cell primary dendrites. Mitral cell somata were strongly labeled, but their axons were negative. Punctate staining was noted on granule cells. Presumptive necklace glomeruli, which receive input from a distinct subset of olfactory receptor neurons, were unlabeled. In the accessory olfactory bulb, immunoreactivity was absent in vomeronasal neuron axons and in glomeruli. However, granule cells and some mitral/tufted cells were labeled. In piriform cortex, staining was prominent in pyramidal neurons of layer II, and was also seen on somata, primary apical dendrites and larger basal dendrites of the pyramidal neurons of layer III. Weak staining was noted in cells of layer IV. In the anterior olfactory nucleus, pyramidal cells were modestly labeled, and, in pars dorsalis sometimes had very long labeled processes extending towards the rhinal fissure. These results provide a firm foundation for further functional analyses using a combination of electrophysiological, immuno-electron microscopic and dynamic imaging techniques.

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72 Poster

DISTRIBUTION OF IGF-IR IN THE OLFACTORY BULB

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Insulin-like growth factor (IGF-I) is involved in the regulation of animal growth and tissue differentiation. The IGF-I receptor (IGF-IR) mediates most of the biological effects of IGF-I. The expression of this receptor is developmentally regulated in the brain, reaching its highest level at late embryonic and postnatal stage. Considering that the olfactory system constitutes a natural model for studies concerning neurogenesis and synapses formation, in the present study we have analysed the spatiotemporal distribution of the IGF-IR in the olfactory bulb (OB) of both developing and mature rats. We have previously shown that a subset of ramdomly distributed olfactory neurons are IGF-IR positive (IGF-IR positive).

Adult rats, postnatal day 1 and day 19 embryos were studied. Serial sections of the OB were immunostained with an antibody against IGF-1R and visualized with DAB.

At E19, all the axons of the nerve fiber layer appeared to be labeled. No staining was observed in the dendritic zone. However, some IGF-IR positive axons seemed to penetrate glomerular-like structures (protoglomeruli).

At P1, the majority of IGF-IR positive olfactory axons seemed restricted to the olfactory nerve layer, but scattered glomeruli were positive throughout the OB.

In the mature OBs, scattered glomeruli contained IGF-IR positive fibers. These glomeruli were found througout the whole OB, although the highest number were located in the middle portion of the OB. The presence of IGF-IR positive fibers in the ONL was also frequently observed.

Here, we describe an unsual distribution of the label in the glomeruli. This distribution shows no pattern and no zonally related expression. It appears to be related with the development and maturation of the OB. However, there still remains the question, why is there convergence of the IGF-IR positive axons on a subset of glomeruli?

73 Poster

EXPRESSION PROFILES OF SELECTED CELL POPULATIONS IN THE MOUSE OLFACTORY BULB

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The qualitative and quantitative analysis of changes in the expression of multiple genes during development and in disease states is of growing interest to both the basic and clinical sciences. In the present work, we examined gene expression in restricted tissue regions and identified neuronal populations in the rodent olfactory bulb (OB). Taking advantage of its laminated structure, in initial studies we microdissected each layer of the OB and compared the expression of various genes and splice variants in each layer by RT-PCR. These genes included various glutamate receptor subunits and subtypes, potassium channels, α1-adrenergic receptors, as well as a number of cell-specific markers. Our results show that the expression of the different genes and splice variants varies in each OB layer. For example, the metabotropic glutamate receptor-1a splice variant (mGluR1a) was primarily expressed in the external plexiform (EPL) and mitral cell layers (MCL), with lower expression levels detected in the glomerular (GL) and granule cell layers (GCL) respectively. Conversely, the mGluR1b splice variant is primarily expressed in GCL, but was also detectable at lower levels in all other layers together with weak expression of the mGluR1d and mGluR1f splice variants. To extend the analysis of single OB layers, we are currently defining the expression of these and other genes in acutely dissociated mitral cells, unequivocally identified by the expression of β -galactosidase in GAD-lacZ transgenic mice. For these experiments we developed protocols for extracting the RNA from small cell numbers, followed by RT-PCR. Data from pools of 25-50 identified mitral cells, as well as from single mitral cells, confirm and extend the results obtained from the microdissected MCL. This molecular approach represents an unique tool to explore genes that underlie function, development and plasticity in specific, identified neuronal populations.

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74 Poster

MATHEMATICAL MODELS OF IONIC DIFFUSION IN OLFACTORY GLOMERULI

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First-order olfactory neuropils characteristically are organized into round glomeruli, which are partially enveloped by glial borders. The effects of this characteristic organization on olfactory information processing are poorly understood. The extracellular concentration of potassium ions ($[K+]_o$) must rise following odor-induced activation of olfactory receptor axons terminating in specific glomeruli. To explore possible effects of such changes on the neural activity within and among glomeruli, we developed a theoretical model to simulate the diffusion of K+ in extracellular spaces of the glomeruli of the moth *Manduca sexta*. Based on light-microscopic examination of *Manduca* glomeruli, glomeruli were modeled as spheres, with receptor axons terminating in one hemisphere and a "mouth" opening onto a non-synaptic neuropil from the other hemisphere. K+ released into the extracellular space by activated axons was assumed to diffuse freely through the narrow extracellular spaces in and between glomeruli. The rates of K+ diffusion within the glomerulus and through the glial envelope were estimated based on measurements from electron micrographs and theoretical analyses of diffusion in inhomogeneous media. The time-dependent diffusion equations were solved in spherical coordinates using a finite-difference method. Our results indicate that the glial envelope forms a significant barrier to the spread of K+ into neighboring glomeruli, thus reducing the likelihood of cross-talk between glomeruli, and may cause long-lasting elevation of $[K+]_o$ to levels that influence neural activity within the activated glomerulus. Such effects could enhance olfactory discrimination and sensitivity, respectively.

75 Poster

CHARACTERIZATION OF A NOVEL SET OF SMALL GLOMERULAR-LIKE STRUCTURES IN THE MOUSE MAIN OLFACTORY BULB

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Plant lectins, carbohydrate binding proteins, label subsets of olfactory receptor neurons (ORNs) in the olfactory epithelium and their axons in the olfactory bulb (Plendl, 1998). In a screen of adult mice, the lectin *Ulex europaeus* (UEA) in addition to labeling a subset of main glomeruli, labeled a set of small, spherical glomerular-like structures. These UEA+ structures most closely resembled nidi, small delimited areas of neuropil in the laboratory shrew (Kosaka and Kosaka, 1999). While UEA labeling in the mouse may prove homologous to nidi, we use the term micro-glomeruli until they are more fully characterized. Micro-glomeruli were similar in size to the glomeruli of the accessory olfactory bulb, ranging from 10-20 μm in diameter. Micro-glomeruli were found throughout the main olfactory bulb, primarily at the juncture of the glomerular and external plexiform layers. It is important to note that micro-glomeruli appeared as discrete anatomical units. While they were often in close proximity to large glomeruli, the neuropil of micro-glomeruli were not contiguous with neighboring glomeruli, as was evident from the unlabeled cell bodies surrounding the UEA labeled neuropil. UEA+ processes resembling axon fascicles were often observed running from the nerve fiber layer and entering micro-glomeruli. Like nidi, micro-glomeruli were OMP negative. However, UEA+ processes were NCAM+ suggesting that micro-glomeruli may contain sensory axons. The UEA+ processes within micro-glomeruli interdigitated with MAP2+ dendrites. UEA+ micro-glomeruli were also synaptophysin positive suggesting the presence of synapses. Micro-glomeruli were not previously recognized in the mouse olfactory bulb, perhaps due to the absence of OMP staining. However, given the heterogeneity of proteins expressed within this neuropil relative to that seen in conventional glomeruli, their discovery raises intriguing questions regarding the potential heterogeneity of sensory receptor cells in the olfactory epithelium and their central targets.

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76 Poster

BILATERAL NEURONS CONNECTING HOMOTOPIC AREAS OF THE TWO ANTENNAL LOBES IN THE FEMALE MOTH HELIOTHIS VIRESCENS

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Both in insects and vertebrates the olfactory pathway from the periphery to higher integration centres is mainly ipsilateral, except for in species of Diptera. Thus, in moths the olfactory receptor neurons target the ipsilateral antennal lobe, transmitting the information to interneurons projecting in the ipsilateral protocerebrum. Bilateral tracts are also described, e.g. the antennal commissure connecting the two antennal lobes. In Manduca sexta this commissure consists of approximately 45 fibres (Homberg et al. 1988). However, knowledge about the neurons forming this tract is scarce in the various insect species; only one neuron described in the honeybee (Iwama et al. 1995). Based on intracellular recordings combined with stainings and confocal microscopy reconstructions, we here present the morpholgy and some response properties of neurons directly connecting the two antennal lobes by the axon passing through this particular commissure. These bilateral neurons targeted olfactory glomeruli in homotopic areas. One of the neurons extended neuronal branches also outside the antennal lobe, in the area of the antennal mechanosensory and motor centre and the ventro-lateral protocerebrum. The neurons responded to antennal stimulation with plant odours, showing inhibitory as well as excitatory responses. These results demonstrate that bilateral information enters the olfactory pathway already at the level of the antennal lobes in the female moth. The results further suggest that integration of multimodal information takes place in some antennal lobe neurons.

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77 Poster

THE EMERGENCE OF COMPARTMENTAL ORGANIZATION IN OLFACTORY BULB GLOMERULI DURING POSTNATAL DEVELOPMENT

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The olfactory bulb glomerulus is a discrete and heterogeneous neuropil where olfactory receptor cell axons synapse with dendrites of mitral, tufted and periglomerular neurons. We and others have shown that the olfactory bulb glomerulus exhibits a distinct heterogeneous, subcompartmental organization. Axonal subcompartments are composed primarily of olfactory receptor cell axons, while dendritic subcompartments are composed primarily of dendritic bundles surrounded by glial processes. These subcompartments are further characterized by their synaptic connections: primary afferent axodendritic and local-circuit dendrodendritic synapses segregate within the glomerulus into axonal and dendritic subcompartments, respectively. To better understand the maturation of glomeruli and the spatio-temporal interactions that occur during the emergence of subcompartmental organization, we employed confocal microscopy and markers for immature and mature olfactory receptor cell axons in parallel with a marker for synaptic structure in developing rats. Sprague-Dawley rats at postnatal days 1, 6, 12, and 18 were processed for single and double label immunocytochemistry for olfactory marker protein (OMP), growth associated protein (GAP-43), and synaptophysin. The appearance of a mature or adult-like subcompartmental organization within the glomerulus emerged by postnatal day 12. Earlier in development immature axons entered the core of the glomerulus and moved to the periphery as they matured. However, beginning around 12 days postnatal, immature axons distributed in the periphery and moved toward the core as they matured. This change in the trajectories of axons into glomeruli suggests that different rules may be followed in establishing versus maintaining glomeruli. Double labeling with OMP and synaptophysin demonstrated strong colocalization compared to GAP-43 and synaptophysin double labeling, which showed much less colocalization, consistent with the notion that OMP is associated with more mature axons.

78 Poster

BMPS AND BMP-ANTAGONISTS IN THE OLFACTORY SYSTEM

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Bone morphogenetic proteins (BMPs) belong to the TGF-beta superfamily of signalling molecules. These factors are involved in early neural development and differentiation, and are implicated in neural plasticity. The variety of BMP functions is, in part, governed by their large number, and ability to homo- and heterodimerize. These ligands are recognized by dimeric receptors with varying subunit compositions. Specific antagonistic proteins with very different properties add to the complexity of this system. In the present study we analyzed the expression of several BMPs and their antagonists in the olfactory system of the mouse during embryonal and postnatal development. We show the expression patterns of BMP4, 6, and 7 by immunohistochemical detection. mRNAs for the specific BMP antagonists Chordin, Follistatin, Noggin and for the Chordin-specific protease Tolloid/BMP1 have been detected by in situ hybridization. The glomerular, mitral cell, and subependymal cell layers show expression of several BMPs and/or BMP-antagonists. We demonstrate that BMP4 and BMP6 are expressed by olfactory receptor cells and show evidence for the secretion of BMP6 protein into the glomeruli. We show the downregulation of BMP antagonist mRNAs in the periglomerular layer following deafferentation. Analysis of the BMP antagonist expression in OCNC1-KO mice suggests that BMP secretion is not dependent upon evoked activity. We hypothesize that the differentiation of glomeruli is influenced by complex interactions between BMPs and BMP antagonists.Supported by NIH grants DC-03112 (FLM), DC00054-01 (DMC), and DC00055-01 (GV), and Univ. of Torino Fellowship (CM).

79 Poster

RADIAL GLIA DEVELOPMENT IN THE OLFACTORY BULB: A ROLE IN GLOMERULAR FORMATION?

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The developmental formation of olfactory glomeruli has been of increasing interest following observations that axons from olfactory receptor neurons (ORNs) expressing the same olfactory receptor gene (ORG) converge onto two or a few topographically fixed glomeruli in the olfactory bulb (OB). Mature glomeruli are multicellular assemblies containing ORN axons, astrocytes, juxtaglomerular neurons and the dendrites of second order mitral/tufted cells. Recent studies in rat have explored the sequence in which these cellular elements are added to glomeruli and suggest that ORN axons and radial glia/astrocytes are the first two cellular elements to exhibit glomerular morphology. The coalescence of mitral/tufted cell dendrites into glomeruli and the addition of juxtaglomerular neurons occurs later. To investigate possible interactions between ORN axons and radial glia during the formation of glomeruli in more detail, we labeled ORN axons and radial glia at 24 hour intervals by immunohistochemistry. In order to examine the structure of individual radial glia we developed a novel method of generating and applying 'nanocrystals' of DiI such that the processes of single radial glia are labeled in the embryonic brain. This study showed that OB radial glia do not form straight parallel structures like radial glia in the neocortex, but rather take a convoluted pathway involving several twists and turns between the ventricle and the bulb surface and consistently extend branches deep to the developing mitral cell layer. The apical processes of radial glia mingle with ORN axons from the earliest detectable stages of glomerular formation (E18 in mouse). These apical processes form highly restricted tufts, or 'glial glomeruli' at the same time that ORN axons are forming 'axonal glomeruli'. The tight spatiotemporal relationship between the glomerulization of radial glia processes and ORN axons during development suggest that radial glia processes play a role in the formation and/or stabilization of mammalian glomeruli.

80 Poster

OLFACTORY BULB PROGENITOR CELLS IN ADULT MICE EXPRESS THE DOPAMINE PHENOTYPE DURING MIGRATION

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Olfactory bulb (OB) dopamine (DA) neurons migrate from the anterior subventricular zone (aSVZ) through the rostral migratory stream (RMS) to the periglomerular (PG) region of the OB even in adult mice. The current studies used immunocytochemical and in situ hybridization techniques to determine where tyrosine hydroxylase (TH), the first enzyme in DA biosynthesis, is first expressed. The studies employed transgenic mice that express a lacZ reporter gene driven by 8.9 kb of TH upstream promoter. Adult heterozygous transgenic mice were perfused and processed for immunostaining of TH, β-galactosidase (β -gal) and CaM kinase IV (CaMKIV) as well as for non-radioactive labeled in situ hybridization of TH mRNA. Cells in the aSVZ or RMS did not contain either β -gal, TH message or protein. β -gal immunoreactive cells occurred not only in the glomerular layer (GL) but also in the mitral (MCL) and granule cell (GCL) layers. Numerous β -gal stained cells, with a granule cell-like morphology, were found in the MCL. In the GL, β -gal immunostaining occurred in all TH-positive cells, but only a subset of the β -gal-positive PG cells displayed TH staining. CaMKIV exclusively stained the nuclei of granule cells that did not express any of the TH markers. In situ hybridization showed that the TH signal was strong in PG cells and weak in granule cells of the MCL. Double labeling demonstrated that TH mRNA and β -gal immunostaining colocalized in cells of the GL and MCL. These results demonstrate that β -gal-containing cells represented a subpopulation of OB cells that expressed TH message in the GL and MCL but TH protein only upon reaching the PG region. The data suggest that some migrating OB progenitor cells begin to express the DA phenotype before they reach their final destination but that full differentiation occurs only in the PG region.

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81 Poster

FUNCTION REGULATES CELL SURVIVAL IN THE DEVELOPING OLFACTORY BULB

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Cell death, an important factor in determining the final cellular composition of the developing brain, is regulated at least in part by afferent input. The olfactory system is particularly well suited for studies of afferent-dependent neural development as it is strictly laminated, has a well-studied synaptic organization, and is easily manipulated. For example, surgical closure of an external naris in neonatal rats increases cell death within the ipsilateral olfactory bulb (Frazier-Cierpial and Brunjes, J Comp Neurol, 289 (3); Najbauer and Leon, Brain Res, 674(2)). However, a systematic examination of developmental and deprivation-induced patterns of cell death has never been reported. The present study used TUNEL to examine cell elimination in normally developing rats, as well as in animals that had a single naris closed on either postnatal day 1 (P1) or P30. Furthermore, reversible naris closure was used to examine the role of sensory function in directly regulating cell survival. TUNEL-positive profiles were high during the first postnatal week, but then decreased with continued development till at least P60. Conversely, permanent naris closure on P1 resulted in dramatic elevations in TUNEL labeling with increasing age. Interestingly, occlusion from P30-60 also resulted in slightly higher levels of labeled profiles. To examine whether cell death was directly linked to olfactory function, occluded nares were reopened on P20. Reestablishment of normal airflow for 10 days completely abolished the deprivation-induced increase in TUNEL labeling. The data provide new information on cell death within the developing olfactory bulb and further emphasize the importance of afferent function in regulating cell survival. Supported by NIH grant DC 00338.

82 Poster

CELL DEATH IN THE OLFACTORY BULB OF ADULT ZEBRAFISH FOLLOWING PERIPHERAL DEAFFERENTATION

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Ablation of an olfactory organ in adult zebrafish results in a significant decrease in volume in the ipsilateral olfactory bulb. We are examining the cause of this phenomenon by investigating potential neuronal loss in the olfactory bulb following the peripheral deafferentation. The zebrafish provides a model in which the olfactory organ is easily accessible for complete removal, the animals easily survive the surgery and recover fully, and the olfactory bulbs are small enough to allow rigorous analysis of the resulting effects.

Unilateral olfactory-organ ablations were performed on anesthetized adult zebrafish using a small-vessel cautery iron. Deafferented and control fish were allowed to survive for various times from 1 hour to 3 weeks after the procedure. After over-anesthetization and paraformaldehyde fixation, brains were dissected and embedded in paraffin. 10- μm sections were cut and mounted onto silane-coated slides. Slides were processed with the TUNEL method, using the Apoptag kit (Intergen). This kit uses an antibody reaction to label cells undergoing DNA-fragmentation, indicating an apoptotic response.

There are two waves of cell death in the olfactory bulb following removal of the primary afferent innervation. The first wave occurs immediately after the surgery and likely represents an immune response. The second wave of cell death occurs at 24 hours after surgery and may account for the decrease in cell number observed several weeks after the surgery. By 1 week, levels of cell death return to control levels, which are minimal. This research begins to explore the influence of the peripheral olfactory organ on the maintenance of the structure of the olfactory bulb in adults. The advantage to these studies in zebrafish is the potential for examining the molecular mechanisms underlying this interaction.

83 Poster

NEURONS GENERATED IN THE OLFACTORY BRAIN OF ADULT DECAPOD CRUSTACEANS: LONG TERM SURVIVAL AND INFLUENCE OF SENSORY INPUT

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In vivo labeling with the S-phase marker BrdU established that proliferation persists in the soma clusters of the central olfactory pathway of adult decapod crustaceans. Double labeling with an antibody against FMRFamide, which immunostains many somata in the MC (medial soma cluster comprized of local interneurons) of the spiny lobster Panulirus argus, demonstrated expression of the neuropeptide and hence neuronal maturation in some of the newly generated cells after a 3-months survival time. Here we report that an antibody against substance P, that does not label any BrdU-positive cells in the MC after a 3-months survival time, does so after a survival time of 14 months proving the neuronal identity of these cells and showing that newly generated neurons in the MC and the LC (lateral soma cluster comprized of projection neurons) of the spiny lobster can survive for more than a year, longer than has been shown in any other animal.

Experiments in which one of the antennules housing the olfactory organ was amputated several weeks after a BrdU-injection revealed that the sensory input affects proliferation in the LC but not in the HBC (soma cluster of the hemiellipsoid body, the target of the projection neurons) of adult shore crabs, Carcinus maenas. Here we ask what effect a reversed order of BrdU-injection and antennule-amputation has on proliferation in the LC and HBC. We amputated one of the antennules, injected BrdU after periods of 27 to 40 days and fixed the crabs one day later. With this treatment no statistically significant lateralized effect on proliferation could be detected in either soma cluster. This result together with the one reported previously indicates that olfactory input excerts an effect on the further proliferation of cells born in the LC but not on the mitoses of precursor cells that initially drive proliferation.

84 Poster

GENETICALLY MARKED MITRAL/TUFTED CELLS IN THE MOUSE OLFACTORY BULB

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In the mouse olfactory system, axons of olfactory sensory neurons synapse with dendrites of mitral/tufted cells in the main olfactory bulb within specialized neuropil compartments called glomeruli. Previously, we have genetically labeled these axons and characterized their projections to the main and accessory olfactory bulbs. Here, we have followed a similar approach to genetically mark all mitral/tufted cells including their dendritic and axonal projections. We chose the peptide neurotransmitter neurotensin (NT) because it was shown to be expressed in mitral/tufted cells in the rat starting at early stages of development through the first two postnatal weeks (Kiyama et al., Neurosci. Letters 128, 1991). We confirmed these findings in the mouse by in situ hybridization. A gene-targeted mouse strain was then generated carrying a modified NT locus that encodes a bicistronic message resulting in co-expression of NT and tau-GFP. This genetic modification allows for the visualization of all NT expressing cells including their processes without knocking out the NT gene.

NT-IRES-tauGFP mice at ages between P1 and P9 show green fluorescence in the bulb corresponding to mitral/tufted cell populations. The lateral olfactory tract is clearly visible in both whole mount preparations and sections. Other cell populations known to express NT, including the hippocampal formation, are also labeled. Thus the NT-IRES-tauGFP mouse line is uniquely suited to study early mitral/tufted cell development and connectivity.

85 Poster

DIFFERENTIAL EXPRESSION OF *X-DLL3* AND *PAX-6* GENES IN THE DEVELOPING OLFACTORY EPITHELIUM OF THE AFRICAN CLAWED FROG *XENOPUS LAEVIS*

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In *Xenopus laevis*, a frog that lives almost exclusively in water, the formation of the adult olfactory epithelium involves embryonic, larval, and metamorphic phases. The olfactory epithelium in the principal cavity (PC) develops from the olfactory placode and is thought to respond to water-borne odorants throughout larval life. At metamorphosis, the PC undergoes major transformations and is exposed to air-borne odorants. Also at metamorphosis, the middle cavity (MC) develops *de novo*. The olfactory epithelium in the MC has the same characteristics as the larval PC and responds to water-borne odorants.

Using *in situ* hybridization, we analyzed the expression pattern of the homeobox genes *X-dll3* and *Pax-6* within the developing olfactory system. The results suggest that *X-dll3* and *Pax-6* genes are both involved in establishing the olfactory placode during embryonic development, the formation of the larval PC during early larval stages, and the formation of the MC at metamorphosis. However, subtle differences in cellular and temporal expression patterns suggest differential involvement for these genes. Early in development, *X-dll3* is expressed in the neural and non-neural ectoderm of the sense plate and in all cell layers of the olfactory placode and larval PC. The expression becomes restricted to the deeper layer (neurons and basal cells) of the PC by mid- metamorphosis. Also at metamorphosis, *X-dll3* is expressed throughout the developing MC epithelium and becomes restricted to deep cells (neurons and basal cells) at metamorphic climax. This expression pattern suggests that *X-dll3* is first involved in the patterning and genesis of all cells forming olfactory tissue, and is then involved in the neurogenesis or neuronal maturation in water- and air-sensing epithelia. In contrast, the restriction of *Pax-6* expression to the olfactory placode, young larval PC, and metamorphic MC, suggests that *Pax-6* is specifically involved in the formation of water- sensing olfactory tissue.

86 Slide

THE ANALYSIS OF ODOR MIXTURES BY HUMANS: EVIDENCE FOR A CONFIGURATIONAL PROCESS

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Humans have a limited capacity to analyze odor mixtures with 3-4 being the maximum. This study investigates the large loss of information about odor identity that occurs in mixtures and aims to determine the information on which identification and failure to identify is based. In Experiment 1, 14 subjects used a selective attention procedure to identify odorants in stimuli consisting of 1 to 4 components. As expected, substantial difficulties were encountered in identifying more than 2 odorants, and chance level scores were obtained for the group for each of the odorants in the quaternary mixture. In Experiment 2, 21 subjects used a profiling procedure consisting of 146 descriptors to describe the odor qualities perceived in the same stimuli used in Experiment 1. The results indicated that for some odorants loss of a major characteristic quality occurred even in binary mixtures, but that many of the features of some odorants remained in the quaternary mixture. Comparison of the data from the two experiments indicated that identification of most of the prominent qualities of an odorant was not necessarily sufficient for identification of the odorant in a mixture. In contrast, the loss of some prominent features did not always result in non-identification. A configurational hypothesis of olfaction, analogous to that for facial and object recognition, is proposed to account for the data and the processes underlying odor identification in mixtures.

87 Slide

PERFORM ANCE EFFECTS OF SUBCONSCIOUSLY PERCEIVED ODORS: THE INFLUENCE OF PLEASANTNESS, FAMILIARITY AND ODOR IDENTIFICATION

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The influence of low concentrations of two odors(lavender and orange) and a non-odorous (control) condition on the performance on two tests (vigilance and calculating ability)was investigated in 293 persons, who were unaware of the presence or non-presence of odor. Each subject performed each of the tests twice under different odor conditions. The results indicated that orange odor had a positive influence on performance in both tests, whereas the results obtained under lavender did not differ from those obtained under the control condition. In the mathematical test men made less errors than women. No gender difference was found in the vigilance type task of the letter counting test. In the mathematical test the error rate was significantly lower in the second session than in the first one. No such learning effect was found in the lettercounting task. Performance was not related to independant measures of odor pleasantness and odor familiarity, which were both more positive in people who could identify the odor than in people who could not identify it. Surprisingly, people who could identify the odors showed a better performance on the vigilance test, independent of the odor condition to which they had been exposed. For the calculating task no such difference between the odor identifiers and non-identifiers was found. The results of the experiment do not support the view that the effects of odors on performance are mediated by the feelings of pleasantness that the odors evoke.

88 Slide

EFFECTS OF ODOR ADMINISTRATION ON OBJECTIVE AND SUBJECTIVE MEASURES OF PHYSICAL PERFORMANCE IN ATHLETES.

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Several recent studies have indicated that the presentation of odors can have both positive and negative effects on the performance of cognitively-based tasks. The present study assessed the effects of odor administration on objective and subjective measures of physical performance. Forty athletes performed a modified 15 minute treadmill exercise stress test under each of four conditions. These conditions consisted of the presentation of one of three odorants (peppermint, jasmine or dimethyl sulfide) or a non-odored control condition via nasal cannula. During testing, objective physiological variables such as pulse, blood pressure, and oxygen consumption were measured. In addition, more subjective measures of work-load, such as the NASA-TLX (a questionnaire that assesses perceived workload for a given task or how hard the task was for them to complete) and the POMS (Profile of Mood States) were administered. No significant effects were found for the objective physiological measures for any odorant. However, peppermint odor significantly reduced perceived physical and temporal workload, effort and frustration. Self-evaluated performance was also greater in the peppermint condition, and participants rated their level of vigor higher, and their level of fatigue lower. Few to no effects were found for the jasmine or dimethyl sulfide conditions. The implications are particularly salient in regards to enhancing athletic performance using a non-pharmacological aid and as an adjunct to athletic training and physical therapy. This research was funded by a grant from the Olfactory Research Fund to the first author.

89 Slide

COMPARISON OF BRAIN ACTIVITY INDUCED BY SENSATION AND IMAGINATION

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Previous studies using PET and functional MRI (fMRI) demonstrated that brain activation occurs not only following actual visual, auditory, or motor activity but also following imagination of these stimuli or activities. The aim of this study was to compare brain activation in response to odor stimuli with the response to the imagination of the same odor by fMRI.

Imaging was performed in 13 healthy volunteers using a 1.5 Tesla MRI scanner capable of echo planar imaging (Siemens, Vision ®). 22 axial images were obtained with a matrix size of 128 x 128 pixels and a slice thickness of 6 mm. Activation maps of those brain areas involved in olfactory processing and the processing of odor imagination were derived using correlation analysis technique (SPM?96b). Stimulus delivery was provided by a specialized olfactometer which allowed rapid delivery of odorants (onset<20 ms) with a defined delivery rate, temperature and humidity. The odorant Eugenol was presented to the left nostril in four 800 ms bursts within an 'ON' period of 48 seconds. This was followed by a 42 seconds 'OFF' period when non-odorous air was delivered. During a second 'ON' period the subjects were asked to imagine the previously smelled odorant. This second 'ON' period lasted for 48 seconds as the first one. This procedure was then repeated four times.

Group analysis revealed similar activation maps in response to stimulation and imagination. In both cases frontal areas were activated bilaterally whereas parietal areas (somatosensory) were activated unilaterally on the right side. The level of activation in the cingulate gyrus was significantly higher for imagination of Eugenol whereas during odor sensation the level of activation was higher in the left frontal operculum.

90 Slide

SPECIFIC AND UNSPECIFIC NOCICEPTIVE CHANNELS IN THE COMMON CHEMICAL SENSE: NEW EVIDENCE FOR POLYMODAL CHEMICAL NOCICEPTORS IN THE TRIGEMINAL SYSTEM

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It has been demonstrated that most odorants stimulate olfactory and trigeminal receptors. Human subjects perceive this as odor, pain and irritation. In experimental animals capsaicin pretreatment eliminates the trigeminal activity indicating that these stimulated receptors are nociceptive. The aim of the current study was to find evidence for the existence and functionality of subtypes of nociceptive channels.

19 subjects participated in the experiments. Menthol in 3 concentrations (0.8, 1.5, 3.4 ppm) and CO2 (70 % v/v) were used for stimulation. Menthol of 0.8 ppm only induced odor sensations, while 1.5 ppm induced odor and cooling, and 3.4 ppm induced odor, cooling and pain sensations. CO2 was always painful. Each concentration of menthol including a zero concentration was presented continuously in a separate session for 15 min following a baseline of 15 min with clean humidified air (36.5 °C, 80% relative humidity). Throughout the session CO2 stimuli of 500 msec duration were administered with an interstimulus interval of 1 min. Following the CO2 presentation intensity estimates of pain (CO2) and odor, cooling, and pain (menthol) were obtained. In addition, the negative mucosa potential NMP was recorded using intranasal electrodes referenced to the nasion.

We found that for menthol stimulation the odor sensation habituated more rapidly than the pain sensation, and that there was no habituation for the cooling sensation. The pain sensation induced by CO2 remained almost constant throughout the session. Only shortly after onset of the menthol stimulation there was a transient dose-dependent sensitization in the CO2 reponses, both in estimates and NMPs.

Referring to our findings that nicotine enantiomers can be discriminated by stereospecific trigeminal receptors, one can conclude that there are several rather independent nociceptive channels in the human trigeminal system. We assume that they contribute in a not yet defined way to the Gestalt of irritating chemicals.

91 Slide

STIMULUS-RESPONSE FUNCTIONS FOR OLFACTORY AND TRIGEMINAL DETECTABILITY: PROBING INTO THE RULES OF CHEMOSENSORY AGONISM IN BINARY MIXTURES

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We are exploring the detection of binary mixtures of chemicals by the olfactory and chemesthetic modalities as compared to the detection of the single components. The endpoints of interest include odor, and two trigeminal responses: nasal pungency and eye irritation. Odor detection is measured in normosmics, nasal pungency in anosmics, and eye irritation in both groups. Employing a two-alternative forced-choice procedure with presentation of increasing concentrations, we built stimulus-response functions for the detectability of the single stimuli via the three sensory endpoints. Based on these data, we then prepare binary mixtures of the two components in varying proportions but where the detectability of each component by itself is known. Finally, by testing the actual detectability of the mixtures we can begin to explore the degrees of detection agonism (or antagonism) that the two substances show across the entire range of detection probability: from chance detection to virtually perfect detection. The results from our first binary mixture: 1-butanol and 2-heptanone lent support, as a first approximation, to the concept of chemosensory agonism, in the sense of dose additivity, between members of binary mixtures presented at perithreshold levels. The members of the presently studied binary mixture, butyl acetate and toluene, where chosen to represent a larger degree of difference in chemical structure between them, to test whether such increased difference would reflect on the degree of agonism for detection observed on the three sensory endpoints.

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92 Slide

PUNGENCY FROM ESTERS: CORRELATIONS WITH PHYSICOCHEMICAL INDICES AND CORRESPONDENCE WITH THE NEGATIVE MUCOSAL POTENTIAL.

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In an investigation of nasal pungency, subjects (n = 10) sought to localize the nostril through which they inhaled various concentrations of nine esters: ethyl, n-butyl, and n-hexyl acetate, ethyl, n-butyl, and n-hexyl propionate, and ethyl, n-butyl, and n-hexyl butyrate. On the realistic assumption that the psychometric functions for localization are adequate surrogates for psychometirc functions for detection, we concluded the following: 1) pungency goes from barely detectable to perfectly detectable with a change in concentration of less than an order of magnitude, and 2) threshold for pungency declines with an increase in the size of the alkyl alcohol group and in the size of the acid group of the ester. From the ethyl to the hexyl compound, threshold declined an order of magnitude irrespective of acid group. From the acetates to the butyrates, threshold declined half an order of magnitude, irrespective of the alkyl alcohol group. The results highlighted the importance of physicochemical factors as determinants of pungency. A solvation equation constructed previously to predict thresholds for pungency from the independent variables dipolarity/polarizability, overall or effective hydrogen-bond acidity and basicity, and the Ostwald solubility coefficient in hexadecane at 25° gave predictions highly consistent with the pattern of the psychophysical results. The correlation coefficient between predicted and obtained values equaled 0.984. Measurements of the negative mucosal potential (NMP), putatively a trigeminally-mediated response, from the septum corresponded well with the psychophysical data. For a criterion amplitude of physiological signal, larger molecules showed greater potency. Those results endorse the conclusion that the NMP has psychophysically relevant meaning as an index of pungency.

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93 Slide

BUTANOL DETECTION AND LATERALIZATION: CONSCIOUS AND UNCONSCIOUS MECHANISMS

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Our aims were twofold: (i) to reestablish whether weak concentrations of butanol were impossible to lateralize, but when sufficiently strong they could be, i.e., above the chemesthetic threshold, and (ii) to determine whether there were concentrations of butanol which could be lateralized, but the judgements of the subjects were based primarily upon guessing, and not upon a clear awareness of the stimulated side. We first determined an ascending, binary, forced-choice detection threshold for butanol. Using a similar procedure in which we presented butanol and a blank simultaneously to both nostrils, we estimated the lateralization threshold, which is higher then the detection threshold. After estimating thresholds, the olfactory detection threshold concentration, one tertiary-step higher, four tertiary-steps lower, and a blank were administered repetitively in random sequence. Subjects determined which one of two stimuli, presented sequentially, was the odor (blank). Subjects also marked their degree of certainty/guessing, on a continuous scale, in each trial. The same procedure was repeated for butanol lateralization (with the concentrations corresponding to the lateralization threshold, one step higher, and four weaker concentrations). The results confirmed that at the level of the detection threshold the subjects were usually correct, but they often guessed, and were not aware of the stimuli. The same occurred for lateralization, although the concentrations necessary for lateralization were quite strong. Thus, as in olfaction where the stimuli are very weak, there appears to be perception of a stimulus that is below the level of conscious awareness; however, in the situation of lateralization, the concentrations of the stimuli are quite strong and readily detectable as olfactory stimuli. Thus it is possible that irritants can have an impact on individuals, perhaps through unconscious mechanisms, at peri-threshold concentrations.

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98 Poster

EARLY TASTE BUD CHANGES INDUCED BY RADIATION DAMAGE

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Radiation therapy for head and neck cancer often results in the loss of the patient's ability to taste. Such sensory deficits can impact the patient's ability to eat and maintain adequate nutritional input. Lack of nutrition can have an adverse effect on the treatment outcome. How radiation damages taste cells, when the damage occurs, and when repair begins is not known. An animal model has been developed to investigate the nature of radiation damage to taste cells. Sprague dawley rats received 6, 12, or 18 Gy of beta radiation from a strontium source, and were examined at 1, 4, 7, 11, or 17 days following the radiation. The number of taste buds was counted with methylene blue stained whole tongues. Histological features were ascertained by light microscopy. At a low dose, the number of taste pores does not change significantly over 17 days. Papillae appear to have a thickened, rim around the pores. At an intermediate dose, only a slight decrease in number of taste pores is noted. Papillae form keratotic rims around the pores. The pore size shrinks. At a higher dose, taste pores decrease in number over the 17 days. Fungiform papillae show marked variation in surface structure, from flat with no pore to tall, volcano-like mounds of epithelial cells. In addition, new papillae can be seen in the regenerating epithelium. These findings suggest that radiation damage to taste cells occurs in a dose-dependent fashion, and that impaired taste function may occur at one of two locations, abnormal pore and abnormal bud.

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99 Poster

TYPE III IP_3 RECEPTORS ARE IN RAT TASTE CELLS

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Taste cells employ a number of different mechanisms to transduce chemical stimuli into neuronal signals. Two important second messengers in taste cells are IP_3 and calcium. In response to saccharin and SC45647, IP_3 and calcium levels in rat circumvallate tissue increase significantly (Bernhardt et al., 1996). Ogura et al. (1997) showed that the increase in intracellular calcium in response to the bitter stimulant denatonium results from the release of calcium from intracellular stores. The effect of IP_3 on calcium levels in other cells occurs after IP_3 binds to receptors located on the membranes of intracellular calcium stores or on the plasma membrane. Three types of IP_3 receptors have been identified, based on derivation from different genes. Type I, type II and type III IP_3 receptors exhibit different affinities for IP_3 and differential distributions in various tissues. In this study we used immunocytochemistry to determine which IP_3 receptor subtypes are expressed in taste cells. Rat tongue sections containing circumvallate papillae were incubated in 10mM sodium citrate, pH 9.0 at 80 °C for thirty minutes to increase antigenicity. Sections were then incubated with antibodies recognizing the different IP_3 receptor types. A subset of taste cells were immunoreactive for type IP_3 receptors while antibodies recognizing IP_3 receptor types I and II showed no apparent labeling. This study suggests that IP_3 type III receptors may mediate the release of calcium in response to sweet and bitter taste stimuli. Our current studies are focusing on whether taste cells immunoreactive for IP_3 type III receptors are also immunoreactive for gustducin.

100 Poster

FUCOSYLTRANSFERASES RELATED TO THE LEWIS-B CARBOHYDRATE EPITOPE IN RAT TASTE-BUD CELLS

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Receptor cells of taste buds are continuously replaced during the life of the animal. During differentiation, new taste receptor cells must acquire: 1) mature morphology with an apical process exposed to tastants at the taste pore; 2) ion channels and/or second-messenger systems for excitation in response to tastants; 3) presynaptic proteins (syntaxin, SNAP-25, and synaptobrevin) involved in neurotransmitter release. The carbohydrate epitope Lewis-b appears on the surfaces of rat taste-bud cells that possess an apical process and contain alpha-gustducin, a G-protein involved in responses to sweet and bitter substances. Lewis-b appears on taste-bud cells that express alpha gustducin and do not express presynaptic proteins, but Lewis-b is absent from taste-bud cells that express both alpha-gustducin and presynaptic proteins. Since both alpha-gustducin and presynaptic proteins should be required for responses to sweet and bitter tastants and for communication with afferent nerves, these observations suggest that Lewis-b is expressed by taste receptor cells prior to synapse formation, and that the carbohydrate group is removed or masked once a synapse has formed. Furthermore, these findings indicate that expression of the glycosyltransferases responsible for synthesizing the Lewis-b epitope is both cell-type specific and developmentally regulated, and suggest a role for Lewis-b in recognition between taste receptor cells and nerves. In humans, synthesis of Lewis-b requires the successive addition of two fucose residues, catalyzed by an alpha(1,2) fucosyltransferase encoded by the FUT2 gene and by an alpha (1,3/1,4) fucosyltransferase encoded by the FUT3 gene, respectively. The homologue of the human FUT3 gene has not been described in the rat. I cloned portions of sequences homologous to FUT2 and FUT3 by the PCR technique from the rat taste-bud library prepared by Dr. N. Ryba, and am currently screening this library to obtain full-length sequences of these genes.

101 Poster

A SUBSET OF POSTERIOR TASTE RECEPTOR CELLS EXPRESSING CCK OR VIP CO-LOCALIZE WITH THE PUTATIVE TASTE RECEPTOR TR2.

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Previous work in our laboratory has demonstrated the presence of the neuropeptides cholecystokinin (CCK), vasoactive intestinal peptide (VIP), and neuropeptide Y in taste buds of rat lingual tissue using immunocytochemistry and RT-PCR. Here we examined whether expression of either CCK or VIP was co-localized with expression of the recently cloned putative taste receptor TR2. Co-expression was examined using a double labeling technique combining immunocytochemistry for peptide visulatization and *in situ* hybridization for TR2 on paraffin-embedded tissue. Immunocytochemistry employed commercial antibodies and the ABC detection technique with diaminobenzidine. TR2 was detected with a 2.5 kb riboprobe using non-isotopic hybridization with an alkaline phosphatase reaction using BCIP/NBT. ICC reaction product was brown whereas *in situ* product was purple-blue. CCK and VIP have similar distributions in taste buds, only a minority of the cells label with cytoplasmically distributed reaction product. The distribution of TR2 in posterior taste cells closely matched that of its original report. Double labeled cells generally visualized with purple reaction product around the nucleus and brown in the more distal process. In VIP double labeling experiments, a total of 142 taste buds were examined. In these taste buds, 320 taste receptor cells (TRCs) labeled solely for TR2, 194 TRCs labeled solely for VIP, and 48 cells were co-localized for both. Of all the cells expressing VIP, about 20% also expressed TR2. In separate experiments with CCK, a total of 476 taste buds were examined. Within these buds, 1052 cells labeled solely for TR2, 1053 labeled solely for CCK and 194 cells co-localized for both. Of all the CCK containing cells, about 15% also expressed TR2. Thus, although comprising a minority, a significant proportion of peptide-containing cells also expressed the putative receptor TR2. This data further implicate peptides as signaling molecules in taste transduction.

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102 Poster

THE ROLE OF CELL CONTACTS IN THE DEVELOPMENT OF AMPHIBIAN TASTE BUDS

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Taste buds comprise the receptor cells of the gustatory sense. Taste receptor cells are unique among receptors cell types in that they do not develop from neurogenic ectoderm, but instead arise from local oral and pharyngeal epithelia (Stone et al, 1995. Barlow and Northcutt, 1995). Experimental results in amphibian embryos indicate that the ability to make taste buds is an intrinsic property of the oropharyngeal endoderm (Barlow and Northcutt, 1997). This finding implies that mechanisms within oropharyngeal endoderm dictate which cells will differentiate as taste buds and which cells will become epithelial. We propose that cell-cell signaling may be important for these decisions, and have developed an in vitro assay to test this idea. Presumptive oropharyngeal endoderm is explanted from neurula stage axolotl embryos nine days before differentiated taste buds appear. Explanted tissue is immediately disaggregated in Calcium-free media then reaggregated in normal media to determine if disruption of normal cell contacts affects the differentiation of taste buds. Reaggregated explants produce differentiated taste buds, and importantly do so in numbers comparable to non-disaggregated control explants. We conclude therefore, that differentiation of taste buds is not disrupted by manipulating cell contacts at an early stage of development. Experiments are currently underway to examine the effects of disrupting cell contacts at later developmental stages to determine if cell-cell communication later in development is important for taste bud genesis. Supported by NIDCD (DC03947 and DC03128 to LAB).

103 Poster

EXPRESSION OF GUSTDUCIN IN THE "GESCHMACKS-STREIFEN" OF INBRED MOUSE STRAINS

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Inbred strains of mice have been shown to differ significantly in their taste responsiveness to bitter stimuli. Immunocytochemical studies show that subsets of taste cells express various molecular markers, including NCAM, gustducin, several of the human blood group antigens, keratin, and other molecules. Fungiform and vallate taste cells of C57BL/6J (B6) mice differ from those of rats in the expression of some of these markers (Christy et al., *Chem. Senses* 24:588, 1999). Although some of the taste buds on the rat's palate (but not the hamster's) are distributed within a strip of epithelium called the "geschmacksstreifen", little is known about the distribution of taste buds on the palate of any mouse strain. We processed fungiform, vallate, and palatal taste buds of B6, SWR/J (SW), and C3HeB/FeJ (C3) mice for immunoreactivity to antibodies against α-gustducin, a G-protein subunit involved in the transduction of bitter compounds. Tissue was cut on a cryostat and free-floating sections were processed for immunocytochemistry. All three strains had taste buds distributed in a line along the anterior border of the soft palate, characteristic of the geschmacksstreifen described in rats. Tissue was examined by confocal microscopy and the number of gustducin-positive cells in 35-mm sections through each taste bud type was determined. The geschmacksstreifen taste buds had similar numbers of gustducin-positive cells in each strain (mean = 8.8) and these did not differ significantly from those in the vallate papilla (mean = 8.0). However, SW mice had significantly fewer gustducin-positive cells in the fungiform taste buds (3.5) than either the B6 or C3 strains (mean = 6.1) and fewer in the vallate (7.0) than the B6 strain (9.1). Although SW mice are more bitter-sensitive than B6 and C3, this behavioral difference is not reflected in a greater number of gustducin-expressing cells.

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104 Poster

THE CKIT RECEPTOR AND SCF REGULATE THE DEVELOPMENT OF A SUBSET OF TASTE CELLS

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ckit is a receptor tyrosine kinase, primarily known for its regulation of cell proliferation, differentiation and migration in melanocytes, germ cells and hematopoietic cells. In preliminary experiments, the ckit receptor was shown to be expressed in adult and developing taste buds. Double immunohistochemistry experiments using antibodies directed against ckit and the G protein alpha subunit gustducin indicated that less than 0.3% of cells inside taste buds were gustducin$^+$ckit$^+$. Because a taste cell expresses gustducin during a large part of its lifetime, it is unlikely that ckit and gustducin are expressed in the same cell at different times during receptor cell maturation; therefore they may be markers for different lineages of gustatory cells.

Using immunohistochemistry, we have determined that the ckit ligand (SCF) is present in adult and developing taste buds. At least one source of SCF in taste buds may be neuronal, since SCF immunopositive fibers are present in taste buds and around adult and developing circumvallate and foliate papillae in the same locations as neurons.

To directly address the possibility that the SCF/ckit pathway is involved in taste cell development, the gustatory papillae of several different ckit (*W*) and SCF (*Sl*) mutant mice were examined. Mice harboring mutations in the ckit receptor (*W/W*^{v}and *W*^{sh}/+) or the SCF ligand (*Sl/Sl*^{d}) survive into adulthood, but have less than 15% of the ckit$^+$ taste cells seen in strain-matched wild type controls. However, at least as many gustducin$^+$ cells are present in the mutant mice as compared to the wild type mice. These results suggest that the ckit receptor is involved in the development of a specific subset of gustatory cells.

105 Poster

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) IS PRESENT IN DIVERSE TASTE CELL TYPES OF ADULT MICE

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BDNF is present in taste bud primordia in the developing tongue and is thought to be the neurotrophin that supports gustatory innervation during development. However, BDNF continues to be expressed in adult mice and is present in some taste cells (Nosrat et al, Development 124: 1333-42, 1997). Since taste cells are constantly renewed throughout adulthood, BDNF may be important for innervation of newly divided taste cells and/or maintaining taste cell innervation. Whether BDNF is present only in newly divided immature taste cells or whether it is present in phenotypically mature cells is unknown. Different taste cell types have been identified based on morphological and immunohistochemical criteria. Morphologically taste cells can be divided into TypeI (dark) and TypeII (light) cells; histochemically many different taste cell types exist. Immunohistochemical markers such as anti-blood group H and gustducin are present in type I and type II cells respectively. In addition, gustducin serves as a marker of cytochemically differentiated taste cells at least 3.5 days old (Cho et al. Chem. Senses 23: 735-42, 1998).

To determine which taste cell types express BDNF and whether mature taste cells express BDNF, we have used immunohistochemical markers including ubiquitin-carboxy terminal hydrolase (PGP 9.5), neural cell adhesion molecule (N-CAM), gustducin and anti-blood group H to identify taste cell types in BDNF^{lacZ} gene targeted 'knock-in' mice. In these mice, the BDNF promoter drives Beta-galactosidase (β-gal) expression. β -gal colocalizes with gustducin, PGP 9.5 and N-CAM in various taste cells. Morphologically, long, slender taste cells, as well as pyriform cells express β -gal. We conclude that BDNF is present in many elongate taste cells, including differentiated type II taste cells, but is absent from basal and edge cells, consistent with the hypothesis that BDNF maintains the innervation of differentiated taste cells.

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106 Poster

TGFβ SIGNALING IN GUSTATORY DEVELOPMENT

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Mammalian lingual taste buds form within papillae. We have shown previously that the genes for the developmental signaling molecule Sonic hedgehog (Shh) and its receptor, Patched (Ptc), are expressed within developing fungiform and circumvallate papillae in mice. Both are expressed broadly in the early tongue but later are restricted to regions in and around developing taste papillae. A similar expression pattern has been observed for another developmental signaling molecule, Bone Morphogenic Protein-4 (BMP4). To elucidate possible signaling interactions between the Shh and BMP4 pathways in papillary morphogenesis, we have compared *BMP4* and *Shh* expression using BMP4/LacZ 'knock-in' mice. Unlike *Shh*, *BMP4* is not expressed broadly in the early (E12) lingual epithelium. By E13, *BMP4* is apparent in circumvallate and fungiform placodes, the same time that *Shh* is localized to papillary regions. *BMP4* is expressed in the developing papillary epithelium, and is coincident with *Shh* in developing fungiform and circumvallate papillae. At late embryonic stages, *BMP4* is expressed in foliate and filiform papillae, whereas *Shh* is never found in these papillae. At E18, *BMP4* expression occurs in a cluster of elongate cells at the center of each fungiform papilla which are covered by a squamous epithelial layer, i.e. where *Shh* expression is also found. These BMP4-positive cells are infiltrated by PGP9.5 positive nerve fibers and may correspond to the cells that will form the taste bud. These results suggest that, as thought for Shh, BMP4 signaling is involved in morphogenesis of taste papillae and taste buds.

107 Poster

TASTE BUD DIFFERENTIATION PRECEDES THE APPARENT DEVELOPMENT OF FUNGIFORM PAPILLAE

Mbiene J. P.[1] [1]*Baylor College of Dentistry-TAMUS Health Center, Dallas, TX, USA*In both Amphibians and Mammals, taste buds arise from either local endoderm or ectoderm. In Amphibians, current data support the hypothesis that taste bud induction is an intrinsic property of the endodermal epithelium; this feature is acquired during gastrulation, and is independent of both neural crest and innervation. However, in Mammals, an experimental test for this hypothesis is not available. Normally in the tongue of rodents, taste buds are restricted to epithelial specializations called gustatory papillae. Current morphological evidence indicates that during development these papillae form before the differntiation of taste buds, and more importantly before innervation. In culture, these papillae form in explanted tongues in absence of innervation but it is not clear whether taste buds develop in the papillae. Given that molecular expression precedes overt morphological differentiation, we hypothesized that the induction of taste buds is concomittant with the patterning of gustatory papillae. To test this idea, we analyzed the expression of keratin 8 in developing papillae in mouse embryos from embryonic day 12 to 15 (E12-15). Anterior tongues were examined for fungiform papillae and taste buds. Surprisingly, by E13-13.5 taste buds were found as discrete keratin spots, containing 6-9 cells, arranged in rows running parallel to the median sulcus of the anterior tongue. Morphologically, at this stage, the tongue is homogeneous with no evidence of fungiform papillae. However, the epithelium is innervated at discrete spots spaced in rows similar to those of keratin expression.We are currently examining whether the differentiation of taste buds is the ultimate step of an intrinsic program of gustatory papilla development or whether taste bud differentiation requires contact-dependent mechanisms with innervation.

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108 Poster

DEVELOPMENT OF PLACODAL NEURONS IN VITRO

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Recent findings in amphibians show that taste buds develop without neural involvement. Thus, developing gustatory fibers must be guided to their appropriate targets in the oropharyngeal endoderm. We aim to test if these fibers use a long-range chemoattractant to locate their target tissue. Toward this end, we developed a culture system to grow neurons that develop from neurogenic epibranchial placodes, neurons that are presumed to innervate taste buds. Prior to the appearance of neurons, placodal ectoderm was removed from salamander embryos and cultured in Matrigel. We find that this gel allows differentiation of placodal neurons and is permissive to neurite outgrowth. Placodal neurons cultured alone have radial, apparently random growth. Placodes were also co-cultured either with target tissue or with non-target tissue and analyzed after six days, when taste fibers have reached the oropharyngeal epithelium in vivo. Our co-culture results thus far resemble results from placodal neurons grown alone; neurite outgrowth is random. However, the pattern of outgrowth varies among individual co-cultures. This likely is due to variable distances between placodal and target tissue in different co-cultures, which is caused by the motile nature of placodal explants. If the target tissue releases a chemoattractant, it may act only over a limited distance. Neurons at too great a distance would not detect the target and random outgrowth is predicted. Thus, we plan to increase the sample size at each distance to determine whether distance between explants impacts the growth of placodal neurons.

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109 Poster

FUNGIFORM PAPILLAE DEVELOP IN INCREASED NUMBERS AND IN ATYPICAL LOCATIONS IN CYCLOPAMINE - TREATED EMBRYONIC RAT TONGUE CULTURES

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Gustatory papillae are arranged on the mammalian tongue in a specific pattern. Among the genes with diffusible protein products that might regulate papilla number and spacing is *sonic hedgehog (Shh)*. We have used teratogenic, steroidal alkaloids that disrupt Shh signal transduction to varying degrees, to learn whether these compounds alter development of papilla pattern on embryonic rat tongue. Tongues from embryos at gestational day 14 (when fungiform and circumvallate papillae first appear) were placed in organ culture as previously described (J. Comp. Neurol. 377:324-340, 1997). Tongues were cultured for two days in a standard medium (STAND); or standard medium with 5 or 10 μM cyclopamine (CYCLOP), an inhibitor of Shh signaling; jervine (JERV), somewhat weaker in disrupting Shh; or, solanidine (SOLAN), a related alkaloid that apparently is not active in disrupting Shh signal transduction. After two days, papilla number and location were determined from scanning electron micrographs. Tongues in STAND, CYCLOP, JERV and SOLAN conditions increased in size and acquired a single circumvallate papilla, and fungiform papillae in a patterned array on anterior tongue. However, average numbers of fungiform papillae on the whole tongue were 95(±16) in STAND and 99(±17) in SOLAN, versus 212(± 23) and 201(±27) in 5 μ M CYCLOP and JERV. On anterior tongue only, tongues cultured in CYCLOP or JERV had about 50% more papillae, and on posterior tongue, about four times as many fungiform papillae, than in STAND or SOLAN. Furthermore, most posterior tongue fungiform papillae were in locations where papillae do not typically develop. Thus, papillae were induced in increased numbers and different locations compared to tongues in standard conditions. These effects of cyclopamine and jervine suggest that interruption of Shh signal transduction might allow lingual epithelium that is normally inhibited to form fungiform papillae.

Supported by NIH NIDCD Grant DC00456 to CMM.

110 Poster

IN VITRO NEUROPHYSIOLOGICAL PROPERTIES OF EMBRYONIC GENICULATE AND TRIGEMINAL GANGLION CELLS: STABLE OVER TIME IN CULTURE, BUT DIFFERENT BETWEEN GANGLIA

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Different sensory ganglia innervate discrete portions of the gustatory organs in the anterior tongue. Trigeminal ganglion cells provide innervation to lingual and fungiform papilla epithelium, but not taste buds. In contrast, geniculate ganglion cells innervate taste buds within fungiform papillae, but not surrounding epithelium. We are using a culture system to study functional differentiation of these ganglion cells during the period when they extend neurites to their target gustatory organs, to learn if developmental changes occur in vitro and to compare properties between these ganglia. Embryos were removed from anesthetized, pregnant rats at gestational day 16, when neurites have reached the papilla and lingual epithelia. Ganglia were dissected, explanted onto matrix - coated coverslips, and maintained in medium supplemented with NGF (trigeminal) or BDNF (geniculate ganglion). After 3 to 10 days in culture, whole cell recordings were made from 254 trigeminal and 150 geniculate neurons. Small, gradual changes in input resistance and membrane capacitance, associated with an increased cell size, were observed during time in culture for both types of cells; however, other electrophysiological properties remained stable. This suggests that these ganglion cells do not differentiate neurophysiologically over several days in culture. When passive and action potential properties were compared between ganglia, geniculate cells had a higher input resistance, more narrow action potential, smaller action potential amplitude and lower threshold of excitation, compared to trigeminal. Also, in all geniculate neurons application of 0.3μ M TTX abolished generation of the action potential, whereas all trigeminal neurons demonstrated TTX-resistant action potentials. About 35% of geniculate cells generated multiple spikes at threshold level; in contrast, all trigeminal cells generated a single action potential. These comparisons suggest that different types and/or distributions of currents determine electrical properties of geniculate and trigeminal neurons at this early development stage.

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111 Poster

TROPHIC FACTORS IN THE DEVELOPING PERIPHERAL GUSTATORY SENSE ORGANS

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Brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) mRNAs are expressed in developing and adult rodent tongue and have been shown to be important for the proper development of the lingual gustatory and somatosensory innervation, and taste bud development in rodents. Distinct, specific, and, in some instances overlapping patterns of BDNF and NT-3 mRNA expression are found in the developing and adult human tongue, gustatory papillae, and taste buds. Neurotrophin 4 (NT-4), another member of the neurotrophin family of neurotrophic factors, plays an important role for the survival of geniculate neurons. These factors have also been shown to elicit neurite outgrowth from cultured cranial ganglia, and BDNF seems to be synaptogenic for BDNF-responsive gustatory fibers. Other growth factors, such as epithelial growth factor (EGF), have been proposed to be important factors for the development of taste buds. Much work has been done in order to understand and characterize the molecules and mechanisms involved in the development of sensory organs for the sense of taste, and there is much work to be done. As it has been agreed upon for almost hundred years, taste buds develop from the lingual epithelium, they are found in predefined and prespecialized areas, and they require interaction with predominantly gustatory fibers for development in mammals, however, not in amphibians.

Different types of organ culture and transplantation approaches can be utilized to study the interactions of the naïve gustatory epithelium and the ingrowing gustatory fibers, some of which I will touch upon in this symposium. In addition, molecular biology techniques, specifically transgenic approaches, will also provide us with strong tools for understanding these interactions in more detail.

112 Poster

THE SACCHARIN PREFERENCE LOCUS (*SAC*) AND THE PUTATIVE SWEET TASTE RECEPTOR (TR1) GENE HAVE DISTINCT LOCATIONS ON MOUSE CHROMOSOME 4

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A putative sweet taste receptor, TR1, has been recently cloned and the TR1 gene has been mapped to mouse distal chromosome 4 (Hoon et al., 1999). The *Sac* (saccharin preference) locus affecting mouse behavioral and neural responsiveness to sweeteners has also been mapped to distal chromosome 4 (Lush et al., 1995; Bachmanov et al., 1997; Blizard et al., 1999). To assess TR1 as a candidate gene for *Sac*, we compared the TR1 cDNA sequences expressed in the tongue of C57BL/6ByJ (B6) and 129/J (129) mouse strains with different alleles of *Sac*. Using TR1 sequence variation between the B6 and 129 strains, we conducted a high-resolution analysis of the chromosomal localization of the TR1 and *Sac* loci in the F_2 hybrids and *Sac*-congenic mice originating from these two strains. The TR1 gene maps proximal to *Sac*, which demonstrates that they are different genes.

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113 Poster

IMMUNOLOGICAL IDENTITY BETWEEN CARBONIC ANHYDRASE VI AND GUSTIN.

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A zinc protein with a molecular weight of 37,000 was isolated (Henkin, 1975) and called gustin due to its possible function in taste. Recently, Thatcher et al. (1998) reported that human carbonic anhydrase (CA) VI is gustin due to the strong identity (99%) between the amino acid sequence of gustin and the deduced cDNA sequence of human CA VI, its zinc content and activity, and its activation of calmodulin-dependent bovine brain PDEase. To re-evaluate whether human gustin is CA VI or not, we performed an immunological investigation. A peptide (93-111 chain of human CA VI) was designed as an antigen for western blotting. The peptide has two active histidine residue sites (94 and 96 of the chain) combined with Zn metal ion and two metal catalytic (111 and 113 of the chain) and no glycosylation sites (67 and 256 of the chain). The antibody raised by immunizing New Zealand white rabbits for the peptide synthesized was used to identify the M.W. 37,000 protein from human parotid saliva.. The protein separated by electrophoresis was transferred to PVDF membrane by an electroblotting technique, and the membrane strip was stained by the antibody using a suitable detecting technique. Only a single, well-defined band of M.W. 37,000 was identified, thus confirming that gustin is CA VI in humans.

114 Poster

MOUSE CYTOCHROME P450 CYP2G1 GENE: PROMOTER STRUCTURE AND TISSUE-SPECIFIC EXPRESSION OF A CYP2G1-LACZ FUSION GENE IN TRANSGENIC MICE

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The aims of this study were to determine the structure of mouse olfactory mucosa-specific Cyp2g1 gene and to identify regulatory sequences important for its tissue-specific expression. A Cyp2g1 genomic clone was isolated from a 129/SvJ mouse BAC library and characterized. The transcription initiation site was localized by primer extension to 16 bases upstream of the ATG start codon. Analysis of a 3.5-kb promoter and 5'-flanking sequence indicated presence of a number of potential recognition sites for known transcription factors, such as the CdxA homeobox factor and the cAMP-responsive element binding protein 1 factor. This 3.5-kb fragment was used to prepare a Cyp2g1-LacZ fusion gene for transgenic mice production. Transgene expression, as determined by beta-galactosidase activity in tissue extracts, was detected in the olfactory mucosa of all five transgenic lines, but not in any other tissues examined, including the liver, lung, kidney, brain, spleen, and small intestine, suggesting that the 3.5-kb fragment contained regulatory elements necessary for olfactory mucosa-specific expression of the Cyp2g1 gene. However, tissue whole-mount staining for beta-galactosidase activity indicated that the expression of the transgene in the olfactory mucosa was patchy in all five lines, implicating the presence of additional regulatory sequences which are necessary for proper expression within the olfactory mucosa.

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115 Poster

OMP TAKES A PARTNER.

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The highly restricted pattern of cellular expression, developmental regulation and phylogenetic conservation of sequence has led to the use of OMP as a hallmark of mature ORNs. Evidence for function derives from altered behavioral and electrophysiological activities of OMP-KO mice. Mechanism has been more elusive. Biochemical data imply an OMP partner. To identify it, T7-phage libraries expressing cDNAs from olfactory neuroepithelium as fusions with phage coat protein, were used in an iterative panning strategy to screen for OMP interactive phage. Phage plaques were picked and their inserts sequenced. All of the phage plaques selected had in-frame inserts, and 90% had identical nucleotide sequences (342 bases). The deduced amino acid sequence matched the first 84 amino acids of a cDNA derived from a member of a small gene family. These cDNAs predict protein molecular weights similar to those we observed as potential OMP partners in radiolabeled gel overlay experiments. Sequence features suggest a membrane association that we are currently evaluating. This would provide a link between cytoplasmic OMP and its influence on plasma membrane events. For this hypothesis to be valid, OMP and its partner must be co-localized. In situ hybridization confirms this for ORNs in both the olfactory and vomeronasal neuroepithelia. To confirm the OMP-partner interaction, three peptides were synthesized that together span 84% of the phage insert sequence. The peptides were individually titrated into samples of [15N]ratOMP and binding monitored by the observation of changes in the 1H and/or 15N chemical shifts of resonances in the 2D 1H-15N HSQC NMR spectra. Only one peptide showed an interaction with OMP indicating the specificity of the interaction. Preliminary fluorescence anisotropy analyses confirm this interaction. These data argue convincingly that we have identified an OMP partner whose characterization will provide insight to the mechanism of OMP function.

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116 Poster

PHARMACOLOGICAL CHARACTERIZATION OF EOG RESPONSES IN CONTROL AND OMP-NULL MICE

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Electroolfactogram recordings (EOGs) performed on OMP-null mice have indicated a role for OMP in the signal transduction pathway. To test whether the effect of OMP depletion was indirect or direct we have constructed an adenoviral vector containing the OMP coding sequence, an internal ribosome entry site (IRES) and enhanced green fluorescent protein (EGFP). EOG recordings from OMP-null animals infected with OMP adenovirus have demonstrated a complete rescue, only 4 days postinfection, suggesting a direct role for OMP in signal transduction pathway.

To determine the site of OMP action we performed a series of pharmacological tests on the olfactory epithelium. Since OMP deficiency has the strongest effect on the recovery phase of the odor response, we tested a number of compounds shown to either activate or inhibit components of a signal transduction pathway thought to be involved in termination of the odor response. Activation of various kinases and subsequent phosphorylation of intracellular components including the odor receptor have been implicated in termination of the odor response. Therefore, we tested the effect of several protein kinase inhibitors. We also tested the effect of protein phosphatase 2A (PP2A) inhibitors, because PP2A has been shown to dephosphorylate odor receptors restoring their initial activity.

A number of compounds, known to affect other events in the signal transduction pathway downstream from the odor receptor, has also been tested to narrow the possible target sites for OMP modulation, including phosphodiesterase inhibitors, membrane permeable activators of cyclic nucleotide gated channels as well as calmodulin and calmodulin kinase II inhibitors. Our data are consistent with an OMP-modulated event being downstream of the synthesis of cAMP.

117 Poster

ODORA CELLS ARE NOT ELECTRICALLY EXCITABLE

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A new model system to study the function of olfactory sensory neurons (OSNs) and the role of odorant receptor proteins in initiating chemosensory signaling has been created from an immortalized cell line derived from the OSN lineage. When moved from undifferentiating conditions (33°C) to differentiating conditions (39°C), these olfactory-derived-odorant-receptor-activatable (ODORA) cells display characteristics similar to mature OSNs *in vitro*[1]. Our study focused on the electrophysiological properties of undifferentiated and differentiated ODORA cells. Undifferentiated cells were cultured at 33°C for a minimum of three days before performing whole-cell voltage-clamp experiments. No inward currents were observed in the undifferentiated cells. Peak outward currents at +60 mV, ranged from 120-3500 pA with an average value of 1022 ± 1042 pA (S.D.; n = 9). Cells differentiated at 39°C were exposed to several culture conditions, and electrical properties were compared. Fetal bovine serum (FBS) concentrations were varied from 0%, 2.5%, 5%, and 10%. The first two concentrations yielded few viable cells while the 5% and 10% FBS supported enough cells for electrophysiological studies. Peak outward currents at +60 mV in cells exposed to 5% FBS ranged from 1186 to 3564 pA and averaged 1918 ± 857 pA (S.D.; n = 6) while in 10% FBS, currents ranged from 373 to 1197 pA and averaged 676 ± 361 pA (S.D.; n = 4). In addition, a retinoic acid derivative, 25 m M TTNPB, was applied for 4 days to differentiating cells. Student t-test comparisons of outward currents from cells grown in FBS compared to TTNPB or to undifferentiated controls showed no significant differences ($P>0.05$). However, outward currents in 5% FBS were significantly larger than those in 10% FBS ($p<0.05$). Small inward currents were observed in a few cells from each 39°C culture condition. None of the culture conditions produced cells capable of generating action potentials.

1. J.Neurosci. 19:8260-8270.

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118 Poster

FUNCTIONAL EXPRESSION OF OLFACTORY RECEPTORS IN CULTURED OLFACTORY SENSORY NEURONS

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Olfactory receptors (ORs) have proven difficult to express in heterologous systems, due to problems that appear to be related to membrane targeting and/or efficient intracellular coupling to generate detectable signals. Olfactory receptor neurons (ORNs) seem to be the most capable cells for expressing ORs. We have extended our approach for functionally expressing olfactory receptors in olfactory receptor neurons (Zhao et al. 1998) by doing this in primary culture. In primary culture, gene delivery is easier than with *in vivo* infection and calcium imaging can be used as a functional assay. We have optimized a culture method (Vargas and Lucero 1999) to make short term ORN cultures which maintain odor responsivity, and this culture is suitable for calcium imaging. Recombinant adenovirus and Semliki Forest virus (SFV) were used to infect the primary cultures and we have observed virus-driven protein expression. Ad-I7-IRES-GFP virus infected cultured neurons have shown responses to the ligands of the I7 receptor in calcium imaging. Compared to adenovirus, the Semliki Forest virus expression system (Berglund et al. 1993) is easier to use and more efficient: viral particles can be produced within two weeks from inserting receptor genes into the expression vector. Therefore SFV-driven OR expression in cultured ORNs may provide a convenient way to study ligand-binding specificity of olfactory receptors.

119 Poster

EFFECTS OF 8-BROMO CYCLIC NUCLEOTIDES ON ION CURRENTS IN CULTURED OLFACTORY RECEPTOR NEURONS OF THE HAWKMOTH *MANDUCA SEXTA*

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A slow and sustained increase of cyclic GMP (cGMP) has been suspected to be involved in adaptation (Ziegelberger et al 1990, J Neurosci 10(4):1217-1225; Boekhoff et al 1993, Insect Biochem Molec Biol 23(7):757-762). In perforated patch clamp recordings from cultured olfactory receptor neurons (ORNs) of *Manduca sexta*, we investigated the influence of the cyclic nucleotide analogues 8-bromo cAMP (8bcAMP) and 8-bromo cGMP (8bcGMP) on ion currents. We used the pore-forming agent amphotericin B, which is selective for monovalent cations, but also passes Ca^{2+} to a certain degree. In the presence of 10^{-8} M tetrodotoxin in the bath solution, we recorded at least four types of K^+ currents. They were voltage-gated, except for one type, which was Ca^{2+}-activated. At least two of the K^+ currents were inactivated by cGMP. A delayed rectifier that has previously been shown to be cGMP-sensitive (Zufall et al 1991, J Neurosci 11(4):956-965) was blocked. Also, the Ca^{2+}-activated K^+ current was observed less frequently after addition of 8bcGMP.

Furthermore, we recorded different types of non-specific cation currents, at least two of which were influenced by the addition of cyclic nucleotides. A TEA-sensitive current with linear current-voltage (I-V) relation and a negative reversal potential (< -20 mV) disappeared after application of both cyclic nucleotides. Another current activated slowly (within seconds) with depolarization and inactivated at the same time scale with hyperpolarization. It was activated by strong depolarization, as well as by 8bcAMP and, less effectively, by 8bcGMP. A third, large current with linear I-V relation, reversed at 0 mV and was observed more frequently after addition of 8bcGMP. We are currently attempting to further distinguish and characterize the different ion channels present in *M. sexta* ORNs. Future experiments with cloned and expressed channels will further facilitate the understanding of the function of individual current components.

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120 Poster

SPONTANEOUS GATING OF THE OLFACTORY CYCLIC-NUCLEOTIDE-GATED CHANNEL

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In the absence of stimuli, isolated olfactory cilia from the Northern grass frog (*Rana pipiens*) have a small conductance to cations. New evidence supports the hypothesis that some of this conductance arises from spontaneous (ligand-independent) gating of the ciliary cyclic-nucleotide-gated (CNG) channels. Ciliary basal conductance, measured between -80 and +80 mV in solutions lacking divalent cations and K^+, averaged 405 ± 52 pS ($n = 31$). Four reagents which inhibit the ciliary current activated by cAMP also inhibited the basal conductance. The reagents (100 μ M W-7, 1 mM amiloride, 300 μ M 3',4'-dichlorobenzamil, and 1 mM *l-cis*-diltiazem) reduced the basal conductance by 46 to 93% (means of 5-8 in each case). In the absence of divalent cations, the cAMP-activated current has a nearly linear current-voltage (I-V) relation. 2 mM cytoplasmic Mg^{2+} and 3 mM external Ca^{2+} reduce the cAMP-activated current at positive and negative potentials, respectively. The I-V relation of the basal conductance was similarly affected by cytoplasmic Mg^{2+} and external Ca^{2+}. It is unlikely that the basal conductance is caused by cAMP retained or produced by the cilium. The cilia have a phosphodiesterase that eliminates the effect of low levels of added cAMP. An upper limit of the channel's mean open probability due to spontaneous gating is 0.03 ± 0.01 ($n = 7$). This value is an overestimate if only part of the basal conductance comes from the CNG channels. Spontaneous gating of the CNG channels is probably one source of background noise and conductance in olfactory receptor neurons.

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121 Poster

BIOPHYSICAL AND PHARMACOLOGICAL ANALYSIS OF OLFACTORY GENERATOR CURRENTS INDUCED BY 'IP3-ODORS?

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Vertebrate olfactory receptor neurons (ORNs) transduce odor stimuli into electrical membrane signals by means of an adenylyl cyclase/cAMP second messenger cascade, but it remains widely debated whether this cAMP cascade mediates transduction for all odorants or only certain odor classes. To address this problem, we have analyzed the generator currents induced by odorants that failed to produce cAMP in previous biochemical assays but instead produced IP3 ('IP3 odors'). We show that, in single salamander ORNs, sensory responses to 'cAMP odors' and 'IP3 odors' are not mutually exclusive but coexist in the same cells. The currents induced by 'IP3 odors' exhibit identical biophysical properties as those induced 'cAMP odors' or direct activation of the cAMP cascade. By disrupting adenylyl cyclase to block cAMP formation using two specific inhibitors of adenylyl cyclase, SQ 22536 and MDL 12330A, we show that this molecular step is necessary for the transduction of both odor classes. To assess whether these results are also applicable to mammalian odor transduction, we examine the electrophysiological responses to 'IP3 odors' in mouse main olfactory epithelium (MOE) by recording EOG responses from the apical surface of the MOE. The results show that inhibition of adenylyl cyclase prevents EOG responses to both odor classes in mouse MOE. These results give added support to previous gene targeting studies (Brunet et al., 1996; Belluscio et al., 1998) and provide evidence that adenylyl cyclase inhibitors can serve as useful pharmacological tools to reversibly disrupt odor transduction in both amphibian and mammalian ORNs.

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122 Poster

MODULATION OF THE VOLTAGE-GATED SODIUM CONDUCTANCE IN MOUSE OLFACTORY SENSORY NEURONS BY FORSKOLIN

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Action potentials in olfactory sensory neurons (OSNs) depend on voltage-gated sodium channels which activate and inactivate upon depolarization. In dissociated mouse OSNs under normal whole-cell conditions, we saw that 7 of 33 OSNs had sodium conductances that were fully inactivated at -70 mV, near the resting potential of these cells. We measured the half-inactivation potential of the sodium current when the intracellular saline contained neither ATP nor GTP at -78.3 ± 7.2 mV (n = 9) (μ±SD). This level of inactivation would significantly depress responses to odor. When the intracellular saline contained 2.5 mM MgATP and 0.5 mM GTP, the potential shifted 10 mV to -67.6 ± 7.0 mV (n = 5). Anomalously negative half-inactivation potentials have been reported in frog (1) and rat (2) OSNs; in frog OSNs a GTP-dependent process has been implicated in regulation of the channels (3). We tested the effect of forskolin, an activator of adenylyl cyclase, on the sodium current. In 3 of 5 cells, 5 micromolar forskolin caused a reversible increase of 31 ± 7% in the magnitude of the sodium current. There was no effect on the half-inactivation potential, but in 2 of 5 cells the activation threshold for the sodium current was shifted negative by 10-20 mV. These data suggest that an endogenous regulatory mechanism may act on olfactory sodium channels to modulate olfactory sensitivity.

1) RYK Pun & RC Gesteland. *Pflugers Arch.* (1991) 418:504-511

2) S Rajendra, JW Lynch & PH Barry. *Pflugers Arch.* (1992) 420:342-346

3) RYK Pun, SJ Kleene & RC Gesteland. *J. Membrane Biol.* (1994) 142:103-111

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123 Poster

THE TIME COURSE OF THE ELECTROOLFACTOGRAM

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The electroolfactogram (EOG) is the summated activity of the olfactory epithelium. We have recorded this response in two preparations: one with direct odor application after surgically opening the nasal cavity and one in which odorized air is pulled into the nose by an artificial sniff. In both preparations, nonpolar odorants (e.g., hydrocarbons) produced larger responses in the ventral and lateral epithelium, while polar odorants produced larger responses in the dorsomedial epithelium. We have argued that there may be intrinsic differences in the sensitivity of the olfactory receptor neurons (ORNs) in these different regions, but that the responses in the intact preparation are further governed by airflow and by the sorption of odorants onto the walls of the nasal cavity. Here we report that the rise times of the EOG are consistent with this interpretation. We compared the times for the EOG to go from 10% to 90 % of maximum for an ester (amyl acetate) and a hydrocarbon (limonene) in both preparations. For a series of odorants in the open preparation, the rise times did vary systematically with electrode position. However, for the intact preparation, the amyl acetate responses developed significantly more slowly at lateral sites than at medial sites. This was not the case for limonene. These results suggest that the spatial distribution of responses to different odorants previously reported for the open preparation may not result solely from differential rates of diffusion through the mucus. However, the sorption of odorants onto the wall of the nasal cavity does contribute to the greater spatial response differences seen in the intact preparation.

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124 Poster

OLFACTORY RECEPTOR CELL SUPPRESSION INDUCED BY CELL DEPOLARIZATION IN BULLHEAD CATFISH

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Negative EOG responses recorded with calomel electrodes to amino acids in brown bullhead catfish (*Ameiurus nebulosus*) indicate that membrane depolarization is the most likely mechanism of olfactory receptor cell (ORN) response. ORNs recorded with Pt-plated, metal-filled, glass microelectrodes are spontaneously active, and the action potential frequencies are in the range of 0.2 to 15.3 Hz. Stimulation by amino acids either decreased (suppressed, 145 cells) or increased (excited, 25 cells) the frequency of action potentials in responsive cells. Both suppression (37% of the suppressed cells) and excitation (43% of the excited cells) were dose-dependent. The cells that responded to amino acid with suppression had significantly higher spontaneous activity than those that responded with excitation. The high correlation between suppressive responses and EOG amplitude ($r_p = 0.98$, $p = 0.007$) suggests that a possible mechanism of ORN suppression and excitation are depolarizing receptor currents. This hypothesis was tested on enzymatically dissociated ORNs in cell-attached configuration. The ORNs displayed action currents typical of spontaneous activity and were electrically excitable. A typical cell (n = 4) triggered most action currents between -50mV and -90mV, whereas one cell displayed the highest action current frequency at ~0mV. Small positive pipette potentials increased the frequency of action currents (excitation) and large positive pipette potentials abolished the action currents (suppression). Negative pipette potentials abolished the spontaneous activity. The action currents were not observed when extracellular Na+ was replaced by choline+, suggesting the involvement of voltage-gated Na+ conductance. Whole-cell voltage-gated Na+ currents activated between -70mV and -61mV and peaked between -43mV and -25mV (n = 9). Steady-state inactivation was complete at potentials more positive than -40mV and half-complete at -83±10mV (x±SD, n = 24). The time constant (τ) of Na+ current reactivation was 40±2ms (n = 2) at -60mV and 20±6ms (n = 3) at -80 mV.

125 Poster

CHEMOSENSORY STIMULI FOR THE CRAYFISH *PROCAMBARUS CLARKII*

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While the chemical senses of saltwater crustaceans have been investigated extensively, comparatively little is known about the physiological properties of chemoreceptor cells found in freshwater crustaceans. Here we evaluated the effectiveness of amines and amino acids, sugars, and bile salts in stimulating chemoreceptor cells present on the second and third pereiopods of a freshwater crustacean, the crayfish *Procambarus clarkii*. Excised pereiopods were placed in an olfactometer that allowed chemical stimulation of the dactyl and propus in one chamber and suction-electrode recording from the exposed nerve in another. Identifiable single cells were rarely encountered, so most of our findings are based on multi-unit responses. Chemosensory fibers were identified based on relative responses to a fish food extract, a mixture of all single compounds tested, and a blank. When applied at a final concentration of ~100 μ M, effective stimuli were maltose, glycine, sucrose, NH_4^+, glucose, and taurodeoxycholic acid (from most to least effective; 22-24 fibers). Entirely ineffective stimuli included glutamic acid, serine, taurine, betaine, lactose, taurocholic acid, and glycocholic acid. Twelve additional fibers were tested with glycine, arginine, lysine, leucine, and hydroxyproline. Only glycine and leucine were highly stimulatory and both compounds stimulated the same fibers to the same degree (at ~100 μ M). Finally, 11 fibers were tested with an ascending concentration series of either glycine or leucine. Thresholds ranged from 10^{-7}-10^{-5} M. The insensitivity of *P. clarkii* to glutamic acid, serine, taurine, and betaine is surprising as these are potent stimuli for many saltwater crustaceans. Also, *P. clarkii* is sensitive to three of the four sugars tested. Carbohydrate sensitivity is relatively uncommon in crustaceans but is established in some intertidal species and other species of crayfish. Sensitivity to carbohydrates appears correlated with dietary preference in these largely herbivorous animals.

126 Poster

CHEMORECEPTOR CELLS AS CONCENTRATION SLOPE DETECTORS

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Antennular chemoreceptor cells of the American lobster Homarus americanus distinguish between different chemical compounds as well as their 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 the lobster lateral antennule to steep (less than 1 s rise time) and shallow (greater than 10 s rise time) odor onset slopes generated by computer-controlled piston pumps or peristaltic pumps. The odor (hydroxy-proline or taurine) was mixed with a dopamine tracer that allowed us to measure the stimulus concentration profile with high spatial and temporal resolution (In Vivo ElectroChemistry.

Average spike frequency increased with steeper onset slopes. 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, single cells could discriminate between a range of stimulus onsets.

127 Poster

ANALYSIS OF OPEN PROBABILITIES AND I-V RELATIONS OF SINGLE CHANNELS IN IDENTIFIED C. ELEGANS CHEMOSENSORY NEURONS

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In nematodes, a number of novel ionic channels have been identified using genetic sequence analysis and cellular expression systems (*C. elegans*) or electrophysiological analysis of intact neurons (*Ascaris*). An interesting complement of channels is expected in these animals because of the unique function of their nervous system: Lacking sodium action potentials, nematode neurons appear to use electrotonic conduction for long-distance signaling. We previously described perforated-patch whole-cell as well as cell-attached and excised-patch single-channel recordings from *C. elegans* chemosensory neurons AWA and AWC. The single-channel recordings provide a random sample of the conductances accounting for whole cell behavior. Single-channel properties qualitatively account for the whole-cell I-V relation. AWA and AWC neurons exhibit very low conductances and few channel openings at membrane potentials near their presumed resting potential but show substantial currents and channel openings at hyperpolarized or depolarized potentials. In an effort to further understand the contributions of various channel types to whole cell properties, we have conducted a quantitative analysis of open probabilities and I-V relations from our sample of AWA and AWC single-channel recordings. Properties of nematode channels recorded in their native environment will also be compared with the behavior of nematode channels in cell expression systems and with the current classifications of ionic channels.

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128 Poster

MAPPING ION FLUX ASSOCIATED WITH THE OLFACTORY SENSILLA OF THE BLUE CRAB, *CALLINECTES SAPIDUS*

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In the euryhaline blue crab we propose that a diffusion-generated ionic/osmotic microenvironment is responsible for sustaining the functional integrity of the olfactory dendrites within aesthetascs (olfactory sensilla) at low salinities. Passive diffusion of ions from the hemolymph to the sensillar lymph via a paracellular pathway is suggested from the findings of previous studies. This diffusion is driven by an actively maintained concentration gradient between the hemolymph and the external environment. To further test this hypothesis, flux levels of Ca^{++} and K^{+} associated with the external surfaces of the aesthetascs were spatially mapped using self-referencing, ion-selective microelectrodes. Animals acclimated to low salinities (both 15% seawater and freshwater) show a net outward flux of ions from these sensilla. The location of maximum flux associated with each aesthetasc conforms to that predicted from structural data (namely, the distal terminus of the constricted region), and corresponds to the permeable section of cuticle separating the olfactory dendrites from the external environment. Maximum concentrations of Ca^{++} and K^{+} measured in the external medium deep within the aesthetasc tuft are well below those present in the hemolymph. These concentrations are interpreted with respect to a potential across the aesthetascs which may limit cation efflux through the cuticle at low salinities.

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129 Poster

IDENTIFICATION OF A NON-AESTHETASC SENSILLUM THAT IS ABUNDANT ON THE OLFACTORY ORGAN OF THE SPINY LOBSTER *PANULIRUS ARGUS*

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Crustaceans detect waterborne chemicals through specialized cuticular sensilla. The olfactory organ of the spiny lobster (*Panulirus argus*) - the antennule - contains many setal types. The aesthetasc sensilla are the best studied of these and have been credited for most olfactory-mediated behaviors. However, recent behavioral studies using the spiny lobster suggest that non-aesthetasc chemoreceptors on the antennules are also important for discrimination of and orientation to food odors. Yet no antennular chemosensilla have been identified besides the aesthetascs. In this study, we describe a putative chemoreceptive sensillum on the lobster's antennule, which we refer to as the 'hooded sensillum'. There are ~500 hooded sensilla on each of the two flagella of the antennule, making it the most numerous setal type after the aesthetascs. These sensilla share morphological characteristics with a previously described bimodal (chemo- and mechano) sensillum, the hair peg organ. Hooded sensilla have a tuft of setules that surround an elaborate central stalk. They are found on almost all annuli (1-9 per annuli) and insert within shallow pits located near the junction of annuli. Using light microscopy, we have identified small clusters of cell bodies at the bases of the hooded sensilla. We are currently using TEM to determine if these cells innervate the hooded sensilla. The predominance and morphological characteristics of the hooded sensilla make them likely candidates for chemoreceptive or bimodal sensilla. Indeed, extracellular electrical activity recorded in response to focal odor stimulation of antennular regions bearing hooded sensilla supports the idea that these are chemoreceptive sensilla.

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130 Poster

BIOCHEMICAL AND MOLECULAR EVIDENCE FOR A PARAMECIUM GLUTAMATE RECEPTOR

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Glutamate is one of several water-soluble stimuli that elicit an attractant response in *Paramecium*. There are at least two binding sites for glutamate on the cell surface. One of these sites mediates the repellent effects of inosine 5'monophosphate (IMP) while another appears to regulate attraction to glutamtate. We are searching for *Paramecium* cell surface proteins that may act as glutamate receptors. A 70 kD peripheral protein has been isolated from a surface protein preparation using affinity chromatography. This protein elutes with both glutamate and IMP and is a candidate for the receptor. We have also used the polymerase chain reaction (PCR) to examine sequences in the *Paramecium* genome that are similar to published glutamate and olfactory receptor sequences from *Caenorhabditis*. We have cloned a 600 bp piece of *Paramecium* DNA that shows similarity to *Caenorhabditis* olfactory receptor sequences and to the sequences of transmembrane proteins from various organisms. When this 600 bp fragment was used as a probe in Southern blotting experiments with *Paramecium* genomic DNA, a 4 kB piece of DNA was visualized. We are using inverse PCR to clone this fragment.

Supported by NIH, the VCC, the University of Vermont, and Pfizer.

131 Poster

ESTIMATING THE NUMBER OF OLFACTORY RECEPTOR NEURONS IN ADULT MALE HAMSTERS WITH THE OPTICAL FRACTIONATOR

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The optical fractionator (West et al., 1991), a stereological tool for counting objects in tissue sections, was used to make unbiased estimates of the total number of olfactory receptor neurons (ORNs) in the olfactory epithelium (OE) of the adult male hamster. Glycol methacrylate sections stained with hematoxylin-azure-eosin were used. Dendritic knobs at the luminal surface, representing mature ORNs, were counted separately from ORN somata. Estimates of total numbers across the receptor sheet resulting from systematic fractionator sampling were converted to surface (linear) density values through measurement of the surface area of the OE. Separate measurements were made on OE segments known from our tract-tracing studies (Schoenfeld et al., 1994) to project mutually exclusively to one of the four quadrants of the main olfactory bulb (MOB). Our preliminary estimates put the total number of mature ORNs (those with knobs) at roughly 8-10 million in each cavity, and the number of all ORNs (number of somata) at 15-30 million overall in each cavity. The surface density of dendritic knobs is about 50,000 per sq mm, a value comparable to numerous estimates in the literature from a number of adult species. Separate sampling of OE segments associated with each MOB quadrant reveals that the surface density of knobs is remarkably uniform across different regions. On the other hand, the density of ORN somata averages about 2-3X the number of knobs and varies widely from region to region, leading to wide variation in OE thickness as well. Roughly half the number of mature ORNs project to the medial MOB, and half laterally. However, proportionately fewer project to the dorsal half of the MOB (only about 25-30%) than to the ventral half, suggesting that there may be far greater convergence onto mitral and tufted cells of the ventral MOB.

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132 Poster

RGS PROTEIN EXPRESSION IN THE OLFACTORY SYSTEM

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Regulators of G protein signaling (RGS) are GTPase activators that modulate signal transduction through G protein-coupled receptors and therefore have an important role in regulation of a variety of cellular mechanisms (Kehrl, J., Immunity, 1998). The presence of RGS2 and RGS3 messenger RNAs and polypeptides were demonstrated in olfactory epithelium by reverse transcription PCR analysis and western blotting, respectively. We used two polyclonal antibodies directed against the polypeptides to determine the distribution of immunoreactivity in paraformaldehyde fixed, frozen rat olfactory epithelium tissue sections. On Western blots, one antiserum (RGS2/3) detects both RGS2 and RGS3. The other antiserum detects only RGS3. The two antisera render a similar but not identical pattern of immunoreactivity. The signal spans the thickness of the epithelium but is particularly dense apical to the supporting cell somata. The apical signal is not continuous along the epithelial surface but alternates with negative patches suggesting that a subpopulation of epithelial cells expresses the target epitopes. In particular, transmission electron microscopy shows DAB reaction product in the sensory neuron cilia of tissue exposed to anti-RGS2/3. Anti-RGS2/3 immunoreactivity is also expressed by some cells in sensory transplant structures following transplantation of olfactory tissue to cerebral cortex. Supported by DAAD05-96-D-7019, FAA97-G-020.

133 Poster

RESPONSES FROM ANTENNAL SENSILLA IN THE ORNATE MOTH TO OLFACTORY SIGNALS

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The Ornate moth, *Utetheisa ornatrix*, uses a complex set of chemical signals to mediate aspects of its reproductive, warning and defensive behaviors. We report here electrophysiological responses from individual receptor neurons on the antenna of male moths to stimulation with graded amounts of the female-produced pheromone, (*Z,Z,Z*)-(3,6,9)-heneicosatriene. Similar neurons are also found in female moths. These results, along with our previous studies, demonstrate the existence of at least three distinct chemosensory systems on the antenna of *Utetheisa*. In addition to the receptor neuron in trichoid sensilla resoponsive to a female pheromone, a second set of neurons found in basiconic sensilla of both sexes, respond to the male-produced pheromone, hydroxydianaidal. Each of these two neuronal classes responds to pheromones involved in either the close-range courtship behaviors elicited by males or the long-range orientation behaviors elicited by females. Distinct from these two sets of pheromone receptor neurons, *Utetheisa* antenna also contain a third set containing two additional olfactory receptor neurons housed in trichoid sensilla of both sexes. These neurons respond to volatile gender-specific odors emanating from male and female animals. These gender-specific odors are chemically distinct from the known reproductive pheromones. They are also, present in various parts of the insect, found in multiple developmental stages and, do not appears to be temporally modulated by behavioral context. Although the significance of the reproductive pheromones is well documented, the chemical composition and behavioral significance of the gender-specific odors is not yet known. We will offer a number of behavioral conditions in which it would be useful for a moth to have the sensory capability to monitor the sex, proximity and density of conspecifics. The existence of these gender-specific receptor neurons suggests the presence of a new complex communication system both within and between the sexes of this insect.

134 Slide

MOLECULAR EVOLUTION OF THE NEMATODE ODR-10 CHEMORECEPTOR SUPERFAMILY OF MORE THAN 800 GENES AND PSEUDOGENES

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The ODR-10 superfamily of chemoreceptors in the nematode Caenorhabditis elegans consists of the str, srd, srh, sri, and srj families plus additional divergent proteins encoded by more than 500 genes, plus about 300 pseudogenes. Their molecular evolution primarily involves gene duplication and diversification. The rapid ongoing pace of this evolution is demonstrated by comparisons with orthologs in the congener C. briggsae, which on average share just 70% amino acid identity. Such comparisons also show that approximately 20% of the C. elegans genes and pseudogenes are newly formed since these two species separated. Balancing this high rate of gene formation is the degeneration of many duplicated genes to pseudogenes, and ultimately their loss through large deletions (indeed several orthologs of C. briggsae genes have been lost from C. elegans). Most of these genes reside on the large chromosome V, and while movements to other chromosomes are relatively common, they have seldom led to formation of new gene lineages. Gene movement within chromosome V is rampant. The superfamily has no ancestrally shared introns, however each of the five major families has 5-8 ancestral introns. These are frequently independently lost within each family, while new introns are occasionally acquired within families. This superfamily comprises perhaps two thirds of the chemoreceptor repertoire of C. elegans, and illuminates both nematode chemoreceptor and genome evolution.

135 Slide

GENOMIC ANALYSIS OF ORTHOLOGOUS MOUSE AND HUMAN OLFACTORY RECEPTOR LOCI INDICATES CLUSTER STABILITY YET MINIMAL CONSERVATION BEYOND THE CODING SEQUENCE

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Olfactory receptor (OR) genes are members of the seven-membrane-spanning, G-protein-coupled superfamily. They reside in large clusters on multiple chromosomes and may number over 1000 in both mouse and human genomes. We have taken a comparative genomics approach in an effort to identify features that may be involved in the apparent rapid evolution of this gene family and in the transcriptional control that results in a single OR gene expressed per cell. We have sequenced 250 kb of a murine chromosome-7 OR gene cluster and used synteny, gene-linkage, and phylogenetic analysis to identify and sequence its orthologous partner in the human genome. Comparison of these orthologous loci indicates that gene content, spacing, and orientation have been maintained. Seven human OR coding sequences were identified in this cluster, all of which have maintained open reading frames at greater than 80% identity to murine orthologs, suggesting functional, as well as genomic stability. This observation contrasts observations made elsewhere which suggest the human OR repertoire is largely pseudogenized. Comparing specific orthologous gene pairs, homology extending through the 5' untranslated regions (UTRs) is evident, suggesting that intron-exon structure has been maintained and that 5' UTR regions may play a regulatory role. Sequence conservation upstream of the start of transcription, however, is minimal or absent among orthologs and paralogs. In general, these regions lack consensus promoter signals or other indicators of common upstream regulatory features. Several transcriptional models are considered, including the possibility that regulation may be encoded within the gene itself, which could have facilitated genomic expansion of the OR family via retrogene, gene conversion, and/or block duplication.

136 Slide

COMMON AND VARIABLE FEATURES IN THE STRUCTURE OF OLFACTORY RECEPTOR GENES.

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An extensive computer analysis of 17 olfactory receptor (OR) genes from cluster on human chromosome 17p13.3 was carried out. It is the first cluster of OR genes for which complete DNA sequence is available. A common gene structure, with an intronless coding region and at least one upstream non-coding exon was predicted by a consensus strategy that we have developed. Potential gene control regions were identified, including specific CT tracts and binding sites for the olfactory specific transcription factor Olf-1. We found that their locations tend to be conserved within a given subfamily of ORs.

The predicted locations of upstream exons for seven transcribed OR genes were confirmed by RT-PCRs using mRNA from human olfactory epithelium. Pairwise comparisons of 5' non-coding exons show that their sequences are conserved between the ORs from the same subfamily but not between the ORs from different subfamilies. Additional unpredicted internal exons were recognized by alignment of the amplified cDNA sequences with the genomic sequences of corresponding OR genes. The existence of such exons (ranged between one and three) signifies variability in exon-intron structure of OR genes. These exons may be spliced in different combinations giving rise to alternative isoforms of OR mRNA. This diversity of OR structure could modulate its translation efficiency. No additional introns were found 3' to the coding regions of OR genes. Repetitive sequences were found to be involved in the determination of OR gene structure: they carry splice sites for non-coding exons and reside upstream from the sites for transcription initiation of some OR genes. The observed structure of OR genes might readily be produced by retropositions that have an important implication in generation of diversity of OR gene families. This event might be followed by OR gene duplications and farther expansion of OR genes repertoire.

137 Slide

MOLECULAR CHARACTERIZATION OF ODORANT RECEPTORS FROM THE FISH

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Environmental stimuli are recognized by sensory neurons and this information is transmitted to the brain where it is decoded to provide an internal representation of the external world. Vertebrates can recognize and discriminate a large number of odorants of diverse molecular structure. How are the tasks of molecular recognition and neural coding accomplished in the vertebrate olfactory system? Individual odorants are thought to activate specific G protein-coupled receptors encoded by a large multigene family. Olfactory neurons are thought to express only one or a few odorant receptor genes. Thus, the problem of discerning which receptor has been activated (and therefore the molecular identity of the odorant stimulus) can be reduced by the nervous system to a problem of identifying which cell has been activated. Neurons with common odorant receptor specificities in turn converge to a small number of glomeruli in the olfactory bulb, suggesting that spatial patterns of innervation in the olfactory bulb are used to encode olfactory information. The logic underlying olfactory coding therefore is a direct consequence of the exquisite selectivity of odorant receptor gene regulation and the concomitant targeting of specific olfactory neurons in the olfactory bulb. As an approach toward identifying ligands for olfactory receptors, we have pursued an expression cloning strategy using the fish as a model system. The odorants that fish detect are water soluble, and include amino acids (feeding cues), bile acids (nonreproductive social cues with possible roles in migration), and sex steroids and prostaglandins (pheromonal cues). Electrophysiological studies have characterized the sensitivities of fish olfactory systems to specific ligands, demonstrating, for example, thresholds for detection in the picomolar (for sex steroids) to nanomolar (for amino acids) range. Thus, the defined properties of odorant-evoked pathways in vivo provide an excellent starting point for the molecular and biochemical characterization of fish odorant receptors.

143 Poster

PERFORMANCE OF DIFFERENT MODELS TO FIT TIME-INTENSITY DATA

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Time-intensity (TI) is an extended methodology to rate duration and intensity of sensory attributes but effort still needs to be made to improve modelling typical TI curves.

We have tested the performance of three models on 288 TI curves from two experiments: a) nine trained subjects evaluated sweetness of sixteen stimuli, four for each sweetener, sucrose, aspartame, D-tryptophan and thaumatin. b) other 9 trained panelists assessed saltiness and pungency of four concentrations of NaCl and KCl. Determination coefficient (R^2), statistic c^2 and the standard deviation of the residuals (Sy.x) were used as criteria for assessing goodness of fit of models to experimental data.

First, a parametric model based on two S-shaped assembled logistic curves was examined (Eilers & Dijksterhuis, 1998). Second, another parametric model where intensity is a continuous function of time was developed, using a set of ordinary differential equations. This function described the latency, the rate of molecular diffusion from the oral receptor areas, the initial concentration of the stimulus, the time where maximum intensity begins to decay and the rate of rinse of the oral receptors. Finally, an equation formally identical to an exponential pharmacokinetic model was applied, considering the oral cavity as one-compartment open model. Rising and falling slopes of the TI curves were modelled by absorption and elimination constants representing the stimulus entry into and exit from the oral compartment, respectively. The TI curves predicted by both parametric models were very close to the experimental TI curves. Otherwise, the kinetic model showed poor fit to the experimental data. The poor performance observed with the kinetic model most probably results from the great number of individual TI curves which showed a delayed lagtime and extensive plateau time.

Eilers and Dijksterhuis (1998). 4th Sensometrics Meeting, Copenhagen, pp. 19-22.

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144 Poster

THE ROLE OF THE RESPONSE CONTEXT IN THE VALIDATION OF INTERVAL SCALING: IMPLICATIONS FOR THE USE OF FUNCTIONAL MEASUREMENT IN THE ASSESSMENT OF TASTE MIXTURE EFFECTS

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A critical assumption behind the use of functional measurement in the study of synergy and suppression effects in taste mixtures is that the responses are measured on an interval scale. There is good evidence that they are, as factorial plots of judged intensity differences among pairs of mixtures and the components yield simple effects that are parallel and linear functions of concentration (e.g., De Graaf & Frijters, 1988; De Graaf, Frijters, & van Trijp, 1987). One question that arises, however, is whether the results from previous studies involving difference judgments generalize to other response contexts. The present study addresses this issue by using magnitude estimation in judgments of intensity of sucrose, fructose, and their mixtures. In a factorial design, participants made magnitude estimates in two response contexts, one in which the components alone were presented along with their mixtures (i.e., mixed context), and the other in which the components alone were presented without mixtures (i.e., unmixed context). Differences in the intensity judgments of these tastants were calculated *post hoc*. This transformation ensures parallelism, but not linearity. Indeed, some deviations from linearity (e.g., polynomial functions in the component trends) were demonstrated, suggesting that the conclusions from studies using difference judgments do not generalize.

145 Poster

RELIABILITY OF INDIRECT SCALING TESTS WITH RESPECT TO THE INTENSITY AND PLEASANTNESS OF SUGAR (IN 4 AND 5 YEAR OLDS)

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Sensory testing with young children is difficult, because they have less cognitive skills and less experience with scaling tasks compared to adults. Therefore the methodology, which is used for adults, is not necessarily useful for young children. Since children in the Netherlands generally not go to school till the age of 4 years, there might be a big difference, in skills to carry out regular sensory tests, between 4 and 5 year olds.

This study investigated whether pair-wise comparison and rank-ordering are useful methods to measure discriminatory ability (analytic task) and preference (hedonic task) for different sugar concentrations in orange flavored beverages, at the age of 4 and 5 years. For the discriminatory ability tests, 5 solutions were used: 7.6, 8.7, 10.0, 11.5, and 13.2g sucrose/100 ml orange beverage. For the preference tests, 5 other solutions were used: 4.8, 6.9 10.0, 14.4 and 20.8g sucrose/100 ml orange beverage.

The subjects of this study were 26 4-year-olds, 45 5-year-olds and 24 21-year-olds. The discriminatory ability and preference were both measured by means of a pair-wise comparison test and a rank-order method. As expected the young adults performed well on all the sensory tests. Only the 5-year-olds were able to perform the analytic rank-order test well [$F(1.67) = 17.9$, $p<0.0001$]. We suggest they were also more able to perform the analytic pair-wise comparison test in comparison with the 4 year olds [$F(1.47) = 3.24$, $p<0.08$]. During the hedonic tests the 5-year olds were also more consistent in their response (Pearson Correlation coefficient between pair-wise comparison test and rank-order test. 4year olds, $r = 0.37$; $p = 0.09$ and 5 year olds, $r = 0.78$; $p = 0.0001$).

In conclusion this study shows that although there is only a one-year difference between 4 and 5 year olds, there is a big difference in doing indirect scaling tests with them.

146 Poster

INFLUENCES OF SEX, AGE, SMOKING HISTORY, AND SELECTED DISEASES ON A STANDARDIZED TEST OF REGIONAL TASTE FUNCTION

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It is now clear that whole-mouth taste tests can be insensitive to major losses of gustatory function, and that regional taste testing is required to accurately assess such function. In this study, we administered one or the other of two versions (72 trial & 96-trial) of the University of Pennsylvania Taste Assessment Test (UPTAT) to 204 men and 260 women spanning a wide age range. In the UPTAT, 15 ul aliquots of intensity- and viscosity-equated stimuli are randomly presented to specific anterior (CN VII) and posterior (CN IX) lingual regions using an Eppendorf pipette. The subject rates the intensity of the stimuli and indicates whether sweet, sour, bitter or salty sensations are percieved. The UPTAT is very reliable (split-half r's for identification and intensity = 0.91 and 0.98, respectively). Four groups of subjects were evaluated: controls and persons who presented to the Smell and Taste Center with chemosensory dysfunction attributible to either head trauma, upper respiratory infections, or nasal/sinus disease. Although data analyses are far from complete, tentative findings are as follows: first, head trauma in particular adversely influences UPTAT scores; second, women, on average, score better than men, particularly in the rear of the tongue; third, older persons perform more poorly than younger ones; and fourth, cigarette smoking has a more adverse influence on sweet (sucrose) and sour (citric acid) perception than on bitter (caffeine) or salty (sodium chloride) perception. In general, the anterior tongue was more sensitive to sucrose, caffeine, and sodium chloride, and the posterior tongue to citric acid. Interestingly, women of all ages who were taking estrogens outperformed their non-estrogen taking counterparts on both the front and the back of the tongue.

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147 Poster

PROP TASTE INTENSITY RATINGS IN NORMOSMIC AND ANOSMIC PEOPLE

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Polymorphism of the bitter taste of thioureas such as phenylthiocarbamide (PTC) has long been the subject of genetic studies. Approximately one-third of humans of European ancestry ('non-tasters') have thresholds exceeding 0.1 mM (Reed et al., 1995). The other two-thirds ('tasters') can detect much lower concentrations of PTC and 6-n-propyl-2-thiouracil (PROP), which is less toxic than PTC. The taster/non-taster distribution differs among racial groups but not between genders. Distributions of intensity ratings for PROP solutions for normosmics and anosmics were obtained from our Taste and Smell Clinic (TASC) Database. Seventy-one normosmic controls (41 ±16 yr) and 390 anosmics (47 ±16 yr) who were normogeusic, as defined by TASC controls, estimated taste intensities of PROP at 0.056mM, 0.18mM, 0.56mM and 1.8mM (Bartoshuk, 1989). These concentrations surround the antimode for bimodal PROP threshold distributions. The total responses of the normosmics to the four concentrations, normalized to responses to tones, fell into two groups: PROP non-tasters with little or no response ($N = 24$) and PROP tasters with substantial responses ($N = 47$). This result is in general agreement with PROP threshold data. In addition, the slope of each subject's PROP response vs. log-concentration curve was computed. The slope measure was highly correlated with the total response measure ($r = +0.86$). The distribution of responses for the anosmics was similar to that for the normosmics. This result indicates that olfaction is not a factor in determining PROP status. Total responses of the normosmics for sucrose, NaCl, citric acid, and quinine.HCl were normally distributed but PROP responses were not ($\chi^2 = 32.1$, $p < 0.0001$). Furthermore, the coefficient of variation for PROP responses (75.1%) was more than twice the average coefficient for the other stimuli (32.9%). We conclude that suprathreshold measures of perceptual intensity for critical concentrations may be used to establish PROP tasting status.

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148 Poster

GENETIC VARIATION IN TASTE: ASSOCIATIONS WITH ALCOHOL SENSATION AND INTAKE

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Heredity appears to contribute to the development of alcoholism. Genetic variation in taste could influence risk of alcoholism through alcohol sensory, hedonic and dietary behaviors. This hypothesis is supported by associations between 6-n-propylthiouracil (PROP) bitterness, one marker of genetic variation in taste, and alcohol sensation (eg, Bartoshuk et al, 1993; Intranuovo and Powers, 1998). Studies (eg, Pelchat and Danowski, 1992; Dicarlo and Power, 1998) also show highest frequency of PROP nontasters in offspring of alcoholics. As part of The Genetic Taste and Dietary Behavior Study, we examined the relationship between PROP tasting and alcohol intensity, liking/disliking, and intake in 52 healthy, young adults (28 males, 24 females) who reported low dietary restraint. PROP threshold was determined with a modified up-down procedure. Subjects used the Green Scale (Green et al, 1993) to rate intensity and liking/disliking of 50% ethyl alcohol applied to the left tongue tip as well as bitterness of PROP (quarter log steps, .032 to 3.2 mM). Sensory responses to alcohol were tested in triplicate, usually over one month. PROP threshold and 3.2 mM PROP bitterness identified 11 nontasters, 25 medium tasters and 16 supertasters. Reported yearly intake of alcoholic beverages was determined from an interviewed Block Food Frequency (1998). Data analyses included Chi square, Pearson correlation, and analysis of variance statistics (significance criterion: p .05). Nontasters were more likely than supertasters to rate alcohol intensity below "strong." Alcohol intensity correlated negatively with alcohol preference. Disliking of alcohol was greater in supertasters than in medium and nontasters combined. Alcohol intake shows a PROP effect; average yearly intake of alcoholic beverages of nontasters (378.2±101.3 SE) was significantly less than either medium tasters (198.4±43.5) or supertasters (122.8±24.9). These findings support the hypothesis that PROP nontasting could influence risk of alcoholism through alcohol sensory response and alcohol intake.

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149 Poster

INCREASED TASTE SENSITIVITY IN PATIENTS WITH RIGHT TEMPORAL LOBE EPILEPSY

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Our previous studies have suggested that the anterior temporal lobe (ATL) is important for gustatory perception and that there is a predominance for taste processing favoring the right hemisphere in humans. In addition to elevations in recognition thresholds and decreased accuracy of suprathreshold intensity estimations, patients with surgical resection of the right ATL for the treatment of epilepsy also consistently rate tastes as more intense. However, in previous studies taster status was not evaluated, thus rendering this result uninterpretable.

In the present investigation we applied tastes to the whole mouth, as well as independently to each side of the tongue in two different locations, and asked Ss to make intensity estimations using the Green Scale, following the procedure outlined by Bartoshuk. Ss were also asked to rate the intensity of PROP to determine taster status. Taster status was then co-varied out of a repeated measures MANOVA, which compared intensity estimations in patients with either left or right ATL resection with a matched control group. Consistent with previous results, patients with right ATL resection demonstrated increased taste intensity estimations compared to the control group. This was true for both discrete locus stimulation and whole mouth stimulation. Additionally, all groups rated tastes applied to the right side of the tongue as slightly more intense than tastes applied to the left side of the tongue. Since taster-status was co-varied out of the analysis, these results suggest that removal of the right ATL results in increased taste sensitivity.

150 Poster

GENETIC VARIATION IN TASTE: ASSOCIATIONS WITH SWEETNESS INTENSITY, SWEET LIKING, AND SWEET FOOD ACCEPTANCE

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Bitterness of 6-n-propylthiouracil (PROP) provides a marker for genetic variation in taste. Supertasters (PROP is exceptionally bitter) also taste a variety of sweeteners as more sweet than do nontasters (PROP is weak or tasteless). Limitations in psychophysical methodologies can hinder revealing these associations (Lucchina et al, 1998). As part of The Genetic Taste and Dietary Behavior Study, we examined the relationship between PROP tasting and sweet intensity and liking/disliking. Thirty-two males and 27 females, who reported low dietary restraint, used the Green Scale (Green et al, 1993) to rate intensity and/or preference for sucrose solutions (5, 10, 20% w/vol), sweet foods that were orally-sampled (3 candies, cake, icing, jellies), sweet foods on a questionnaire, and bitterness of PROP (quarter log steps, .032 to 3.2 mM). Ten sweet dislikers (8 females, 2 males) and 31 likers (13 females, 18 males) were identified from hedonic responses to increasing sucrose concentration. Data analyses included the Chi square and Pearson correlation statistics (significance criterion: p .05). Sweet dislikers were more likely than likers to rate 20% sucrose and 3.2 mM PROP as above "strong." In all subjects, average sweetness of 5 sweet foods was also highest in those who tasted PROP bitterness as above "strong." Liking/disliking of sampled and questionnaire sweet foods showed similar associations with PROP and sex. In females, liking for sweet foods fell as PROP bitterness increased; males showed no response or an opposite response. In summary, proper scaling techniques can reveal associations between genetic variation in taste and sweet intensity. The preference results confirm earlier findings (eg, Duffy et al, 1995). In animals, sweet behaviors are influenced by estradiol and mediated by cholecystokin (Geary et al, 1994). It is uncertain how hormones interact with genetic variation in taste to affect sweet behaviors in humans.

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151 Poster

VALID ACROSS-GROUP COMPARISONS: SUPERTASTERS PERCEIVE THE MOST INTENSE TASTE SENSATIONS BY MAGNITUDE MATCHING OR THE LMS SCALE

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One goal of modern psychophysics is to compare perceived sensory intensities across groups of interest. Genetic taste variation permits an assessment of magnitude matching and the LMS scale, methods designed to do this. Magnitude matching (Marks and Stevens) directs subjects to estimate perceived intensities of tastes and sounds on a common scale. Assuming no systematic association between taste and audition, average perceived sound intensities would be the same for groups with varying taste abilities. Expressing the bitterness of PROP (6-n-propylthiouracil) relative to the loudness of sound would permit absolute comparisons of bitterness across groups. The LMS scale (Green and colleagues) was derived by asking subjects to estimate perceived intensities of a variety of oral sensory experiences along with intensity adjectives. Of special importance, they assigned ratings to "the strongest imaginable" oral sensation. This resulted in a line with intensity adjectives located at empirically derived locations: zero at the bottom of the scale, "strongest imaginable" oral sensation at the top. Since oral sensations do not appear to be equivalent to nontasters, medium tasters and supertasters, we modified the instructions. We asked subjects to consider "strongest imaginable" to be the strongest sensation of any kind; Borg suggested that this might be equivalent to all. If it were, then the LMS scale would be able to provide meaningful and consistent measures of perceived intensity. One hundred subjects rated PROP, quinine, sucrose, citric acid and NaCl by both methods. For each method, subjects were divided into three groups by PROP ratings: 25% lowest (nontasters), 25% highest (supertasters) and 50% intermediate (medium tasters). Ratios among the average ratings of the stimuli for each group were similar for both methods: supertasters perceived the greatest and nontasters the least intensities. This convergence supports the assumptions underlying both methods.

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152 Poster

BITTER-SWEET AGE, SEX AND PROP (6-N-PROPYLTHIOURACIL) EFFECTS: A ROLE FOR MENOPAUSE?

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Age related declines in bitterness are generally accepted; age related declines in sweetness are more controversial. We present data that confirm a decline for bitter but not for sweet. Lecture attendees (F = 1384, M = 983) rated the bitterness of PROP paper (pieces of filter paper 3 cm in diameter impregnated with 1.6 mg PROP) on the Green scale (LMS). This adjective-labeled scale was anchored by "no sensation" on the left and "strongest imaginable sensation of any kind" on the right. A subset of attendees (F = 558, M = 339) rated the sweetness of commercially produced candy (Stop and Shop butterscotch buttons) prior to tasting the PROP.

Participants were grouped by age (decades) and perceived bitterness of PROP: lowest 25 % (nontasters), middle 50% (medium tasters) and highest 25% (supertasters). ANOVAs showed that PROP bitterness declined with age differentially for men and women. For men bitterness declined monotonically from the twenties through the sixties. For women bitterness was age stable until declining precipitously in the sixties. The differences were apparent for nontasters, medium tasters and supertasters and suggest a protective role for female hormones prior to menopause. Since bitterness is thought to warn against poisons, preservation of the ability to taste bitter might serve to prevent fetal poisoning during childbearing years. However, not all bitters are toxins (e.g., some bitter constituents of foods are cancer preventives). Thus alterations in the ability to taste bitter across age may have implications for disease risks. ANOVAs showed that sweet taste, unlike bitter taste, was uniformly age-stable for men and women across all taster categories. Although there were no ageeffects for sweet, there were significant sex and PROP effects. Women perceived greater sweetness than did men; supertasters perceived greater sweetness than did medium or nontasters.

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153 Poster

DIFFERENTIAL PERCEPTIONS OF INTENSITY FOR THE FOUR BASIC TASTE QUALITIES IN PROP SUPERTASTERS VERSUS NONTASTERS

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The goal of this study is to compare the perceived strength of tastes of sour, bitter, salt and sweet for subjects who are supertasters versus nontasters of PROP (6-n-propylthiouracil). 100 subjects rated taste intensities for each of four different concentrations of citric acid, quinine, NaCl, and sucrose using two different psychophysical measurement techniques, magnitude matching (MM) and labeled magnitude scaling (LMS). Prior research has shown that PROP tasting status influences the perceived intensity of other taste qualities, however, none of these earlier reports have addressed the relative strengths of perceived taste intensities across all four domains with the same subjects. Bartoshuk and colleagues (2000) have described the methods used in this study and demonstrated general agreement for both ratings of taste intensities. All concentrations were presented in unmarked cups (blindly) to subjects in random order. Our results are based on the categorization of subjects by PROP ratings: 25% lowest (NT = nontasters), 50% intermediate (MT = medium tasters) and the 25% highest (ST = supertasters). The results were unchanged when the analysis was restricted to those subjects (N = 58) that fell into the same groups by both the MM and LMS ratings. At the highest concentrations of each tastant, supertasters rated the intensities stronger than nontasters ($p < 0.001$). Similar results were found for two (logarithmic) dilutions of tastant concentrations. After the third dilution, convergence was attained for the weakest solutions of salt and sucrose. At all concentrations, ST rated quinine as much more bitter than either MT or NT. Differential intensity ratings for ST were strongest for quinine, intermediate for citric acid and salt, and weakest for sucrose. However, MT and NT rated citric acid as strongest, quinine and salt intermediate, and sucrose weakest. These findings have implications for food preferences and perhaps also for clinical complaints.

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154 Poster

THE EFFECT OF COMPOUND-SPECIFIC SENSITIVITY AND CARRY-OVER EFFECTS ON BITTERNESS PERCEPTION

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Investigation of bitterness perception is complex because many factors influence its perception and the mechanisms of taste transduction are only beginning to be understood. Two important factors which have not been studied and are very important in the development of better methodologies to measure bitterness intensity are subject's differential sensitivity and compound specific carry-over effects. Hence, 3 experiments were carried out to study them. The first experiment explored the nature of a relationship between PROP status and the sensitivity to unrelated bitter compounds. The second experiment explored the relationship between thresholds and intensity ratings for PROP and 6 other bitter compounds. The third experiment quantified carry-over effects of the 6 bitterants. Thresholds for PROP, caffeine, denatonium benzoate, limonin, naringin, quinine and SOA were determined for 40 subjects (Exp. 1), who were categorized as PROP supertasters, tasters and nontasters. Twenty-six Ss rated bitterness using Time Intensity (T-I) methodology (Exp. 2). A significant correlation was found between PROP taster status and thresholds for caffeine, naringin and SOA but only for women. Bitterness maximum intensity of the 6 bitter compounds did not vary as a function of PROP taster status. Subjects perceived intensity of the bitterants differently. By cluster analysis, one group of subjects showed bitterness of naringin higher than the other compounds. Another group rated caffeine higher and a third rated quinine higher. For assessment of carry-over effects (Exp. 3), equi-bitter concentrations of the 6 bitterants were determined for each of twelve subjects. Bitterness of 36 paired combinations was rated by T-I. The degree of sensitization and susceptibility to sensitization were compound specific. Caffeine increased the bitterness of the compound presented in the second position by the largest amount, while it was least affected. Regardless of the compound served in first position, bitterness of quinine and denatonium increased most.

155 Poster

INDIVIDUAL DIFFERENCES IN BITTER TASTE PERCEPTION OF SACCHARIN AND ACESULFAM-K

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Bitterness responses to the intensive sweeteners, saccharin and acesulfam-K were studied in relation to PROP taster status. Wide individual differences were observed to the bitter side tastes of saccharin and acesulfam-K at 8% sucrose intensity levels (N = 73). Some evidence of a sour bitter confusion was evident among the naive panelists. However, the bitterness responses to sweeteners were uncorrelated to PROP responses or PROP taster status. Factor analysis (principle components) found that bitter responses to the sweeteners and PROP bitterness responses loaded on separate factors. Saccharin bitterness and Acesulfam bitterness ratings were correlated, $r = +0.52$, $p < .05$. In contrast, correlations of the sweetener bitterness ratings with repeated PROP bitterness ratings ranged from $r = -0.04$ to $+0.15$ (not significant, in the same range as PROP correlations with NaCl saltiness). This suggests a taster/nontaster dimorphism for the bitter properties of these two intensive sweeteners, but one that is a separate mechanism from the bitter transduction mechanism for PROP, especially at higher intensity levels.

156 Poster

TASTE MIXTURE INTERACTIONS AS A FUNCTION OF PROP TASTER STATUS

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Mixtures of dissimilar tastes typically show mutual, but asymmetrical, suppression of the intensity of the individual components. It has been assumed that such interactions are invariant properties of the human psychophysical response to taste mixtures. However, recent research has demonstrated that the intensities of individual tastants vary between individuals as a function of genetic variations in taste receptor density, as indexed by the perceived bitterness of 6-n-propylthiouracil (PROP). We carried out experiments to determine if these variations in taste perception also influence taste perception in mixtures. Subjects were divided into super-, medium-, and non-tasters based on their ratings of the bitterness of a solution of 0.032 M PROP. Two mixture experiments will be reported: sweet-bitter (sucrose/QHCl mixtures), and sweet-sour (sucrose/citric acid) combinations. In each experiment, subjects received factorial combinations of 4 levels of each tastant (including 0). Subjects evaluated the taste intensities, as well as overall mixture intensity. In both experiments, ANOVA found strong main effects and interactions related to the taste components, consistent with previous research. In general, each tastant in both pairs suppressed the intensity of the other, although suppression was asymmetric. In Exp. 1, there were no significant differences between taster groups in their ratings of bitterness or sweetness alone. Group differences were apparent, however, in the impact of QHCl on the sweetness of the mixtures. Non-tasters failed to show the suppression of sweetness by 0.036 mM QHCl shown by medium- and super-tasters. Super-tasters also showed a greater influence of QHCl in determining the overall intensity of the QHCl/sucrose mixtures. In Exp. 2, there was no impact of taster group on ratings of sweetness or sourness, or on interactions in the sucrose/citric acid mixtures. These results are discussed in terms of their implications for PROP sensitivity and perception of taste qualities in foods and beverages.

157 Poster

COLD-INDUCED TASTE PHANTOMS

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We recently reported that thermal stimulation of gustatory areas of the tongue can cause sensations of taste. This phenomenon ('thermal taste') indicates that temperature can have a direct excitatory effect on the gustatory system. We have now found that temperature can induce taste sensations in a second, less direct way: repeatedly cooling the tongue tip to a very cold temperature (5°-10°C) over several minutes can induce 'phantom tastes' in the back of the mouth. 'Cold-induced phantoms' (CIPs) are not experienced by all subjects and their characteristics are idiosyncratic. However, preliminary experiments with three sensitive subjects (conducted with an 8mm X 8mm peltier thermode applied to the midline of the tongue tip) indicate that CIPs (1) tend to be localized to the soft palate and/or to the lateral-posterior tongue (circumvallate region); (2) can be unilateral or bilateral; (3) take minutes to develop and can persist as long as 30 min after cooling has ended; (4) have perceptual qualities that include (but are probably not limited to) salty, sour, and metallic. CIPs appear similar to taste phantoms that were reported in 40% of subjects who had been treated with a topical anesthetic on the anterior 2/3 of the tongue (Yanigisawa et al., Physiol. Behav., 1998, 63: 329-335). Yanigisawa et al. hypothesized that the phantoms occurred when the anesthetic disrupted a tonic inhibitory interaction between the chorda tympani and glossopharyngeal nerves (Halpern, B.P. and Nelson L.M., Am.J.Physiol. 1965, 209: 105-110). By this reasoning CIPs may be induced when extreme cold temporarily renders chorda tympani (and possibly trigeminal) neurons nonfunctional (i.e., cold block). Research is continuing in an effort to learn more about the incidence and characteristics of CIPs and to explore their potential as an experimental model for taste dysguesia associated with peripheral nerve damage.

158 Poster

MAPPING THE TACTILE AND THERMAL PROPERTIES OF THE INTRA-ORAL SURFACE

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The differential cutaneous sensitivities across locations have been mapped for much of the body surface (Martinez et al., 1997). However, there have been few attempts made to map the sensitivity of the intra-oral surface.Von Frey hairs were used to determine punctate thresholds across six intra-oral locations revealing significant differences; cheeks being the most sensitive, lower gingiva the least.A purpose build 1cm2 Peltier thermal stimulator was used to obtain warm sensation (WS), cold sensation (CS), hot pain (HP) and cold pain (CP) thresholds on four intra-oral sites and a glabrous skin site - thenar. Tongue and thenar displayed a significantly lower threshold for both WS and HP than the hard palate, gingiva and buccal mucosa. The sensitivity of the hard palate to CS and CP were significantly less than that of the other sites. Each site was found to be more sensitive to cold than to warm stimuli. Gustatory afferent fibres respond to thermal and chemical stimulation (Green, 1999). Thermal stimulation of the anterior tongue tip induced taste sensations. Rapidly warming the tongue from baseline (30°C) to 45°C induced sensations of sweetness whilst rapidly cooling the tongue to 7°C produced sensations of saltiness in some subjects. The relevance of these findings to intra-oral sensory processing will be discussed.

159 Poster

RINSING WITH CHLORHEXIDINE DEGRADES HUMAN TASTE-STIMULUS IDENTIFICATION

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Rinsing with chlorhexidine, a bis-biguanide antiseptic, reduces the perceptual intensities of NaCl, KCl and quinine.HCl but does not affect the tastes of sucrose, sodium glutamate, or citric acid (Gent et al., 1999). A taste confusion matrix (TCM) was used to measure effects of an oral rinse containing 1.34mM chlorhexidine digluconate on taste-stimulus identification in humans. Ten replicates of 10 stimuli (water, 0.1M NaCl, 0.1M KCl, 0.1M Na-glutamate, 0.3M sucrose, 3mM citric acid, 0.1mM quinine.HCl, and mixtures of sucrose-NaCl, sucrose-citric acid and sucrose-quinine.HCl) were presented to 18 subjects (mean age 33.5 yrs) for identification from a list of 10 stimulus names. Prior to testing, half of the subjects rinsed with water and half with chlorhexidine. Patterns of correct/incorrect responses, and, in bits of information transferred, performance consistency (T_{10}) and pairwise stimulus discriminablility (T_2) were computed. Percent correct for stimuli whose perceptual intensities were reduced by chlorhexidine was 35.1+4.8% for the chlorhexidine-rinse group vs. 74.2±5.5% for controls (XXX0.0001). Group performance for the other stimuli did not significantly differ. T_{10} was 2.02+0.11 bits for the chlorhexidine-rinse group and 2.73±0.11 bits for the controls ($p < 0.0001$). In contrast to controls, T_2 approached chance levels ($T_2 = 0.40$ bit) for the chlorhexidine group for pairwise comparisons of NaCl, KCl, and quinine; and for sucrose-quinine compared to sucrose-NaCl. This suggests NaCl, KCl and quinine tasted very similar following chlorhexidine treatment. T_2 for pairwise comparisons of water and NaCl, quinine or KCl; and sucrose and sucrose-NaCl or -quinine mixtures were also near chance levels for the chlorhexidine group. The rinse with 1.34mM chlorhexidine made NaCl, KCl and quinine difficult to distinguish from water or, in the context of the sucrose mixtures, from 0.3M sucrose. Our study provides additional evidence that chlorhexidine interferes with salt and bitter transduction in humans.

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160 Poster

IMPACT OF CHLORHEXIDINE ON HUMAN TASTE PERCEPTION

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Chlorhexidine, the oral-antiseptic rinse, decreases the salty taste intensity of NaCl and the bitterness of quinine when used chronically, but whether it also selectively blocks taste acutely is unknown. To evaluate this, the impact of brief, oral, chlorhexidine rinses on the taste intensity and quality of eleven stimuli was examined. For each individual tested, all stimuli were first matched for overall intensity so the effects of chlorhexidine would be directly comparable across compounds. As a control treatment, the bitter taste of chlorhexidine digluconate (0.12%) was matched in intensity to quinine HCl, which was found to cross-adapt the bitterness of chlorhexidine. Subjects participated in four experimental conditions: a pre-test, a quinine treatment, a chlorhexidine treatment, and a post-test condition, while rating total intensity and five taste qualities in separate test sessions. Relative to the quinine treatment, chlorhexidine was found to decrease the salty taste of NaCl and KCl, the bitter taste of urea, Sucrose Octa-Acetate, and quinine, and not the tastes of sucrose, MSG, citric acid, HCl, NH_4Cl and the taste of water. Acute chlorhexidine rinses are the first treatment to selectively reduce human perception of saltiness. Chlorhexidine is a symmetrical bis-bi-guanidinium containing compound. The guanidinium group has been involved with several sodium channel blockers including blockers of epithelial (amiloride HCl) and voltage-sensitive sodium channels (tetrototoxin (TTX), saxitoxin (STX), μ -connotoxin (CTX)). The tendency of guanidinium groups to block sodium channels might account for chlorhexidine's inhibition of salty taste in humans. The bitter inhibition is likely to occur via different action. Chlorhexidine is known to alter membrane bound enzymes in bacteria. Perhaps certain membrane-bound proteins, necessary for bitterness transduction, are rendered ineffective by chlorhexidine. Future research directions will be discussed.

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161 Poster

DIRECT EXCITATION OF MITRAL CELLS BY ACTIVATION OF ALPHA1-ADRENERGIC RECEPTORS IN RAT OLFACTORY BULB SLICES.

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The main olfactory bulb receives a significant modulatory noradrenergic input from the locus coeruleus. Previous in vivo and in vitro work showed that norepinephrine (NE) inputs increase the sensitivity of mitral cells to weak olfactory inputs. However, the cellular basis for this action of NE is still poorly understood. The goal of this study was to investigate the effect of NE and adrenergic agonists on the excitability of mitral cells, the main output of the olfactory bulb, in horizontal brain slices. In whole-cell patch clamp recordings, NE (30 μ M) depolarized mitral cells in current clamp (3 - 6 mV), and induced an inward current (10 - 40 pA) in voltage clamp in all cells tested. Phenylephrine (PE, 10 μ M) mimicked the effect of NE and did not change the amplitude of excitatory postsynaptic currents evoked by olfactory nerve shocks. The inward current induced by PE persisted in the presence of TTX (1 m M), and blockers of excitatory and inhibitory fast synaptic transmission (gabazine 5 μ M, CNQX 10 μ M, APV 50 μ M). In these conditions, there was no effect of the beta-adrenoceptor agonist isoproterenol (10 μ M) nor the alpha2-adrenoceptor agonist clonidine (3 μ M). The current-voltage relationship in the absence and presence of PE indicated that the current induced by PE tended to decrease, but did not reverse in polarity, at the equilibrium potential for potassium ions. Our results indicate a direct alpha1-adrenoceptor-mediated excitatory effect of NE on mitral cells. This action appears to be due to a decrease in a leak potassium conductance. We propose that the increase in the response of mitral cells to weak olfactory nerve shocks after activation of the locus coeruleus in vivo could be due at least in part, to direct excitation of mitral cells by NE.

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162 Poster

A PERSISTENT SODIUM CURRENT GENERATES UP-STATE PLATEAU POTENTIALS AND ACTIVE SUBTHRESHOLD RESPONSES TO OLFACTORY NERVE (ON) INPUT IN MITRAL CELLS OF THE MAIN OLFACTORY BULB (MOB).

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Mitral and tufted cells are the principal output cells of the MOB. Mitral cells are bistable. They maintain two levels of membrane potential: an up-state (about-50 mV) at which action potentials and voltage oscillations occur, and a down-state (-60 to -65 mV), subthreshold for spike generation. In the up-state, mitral cells respond to ON input with short-latency spikes. In the down-state, a single ON input, or a brief depolarizing pulse, initiates an exponential depolarization to the up-state, followed by long-latency spikes. The response to ON stimulation is abolished by NMDA/AMPA receptor blockade, but generation of the up state following a depolarizing current pulse is not. We are investigating the currents involved in generation of the up-state using whole-cell recording in rat and mouse slice preparations.

Mitral cells show increasing membrane voltage responses to injected current at membrane potentials positive to about -60 mV. Apparent membrane resistance changes markedly between -60 and -50 mV, increasing 70% during transition from the down-state to the up-state. In the presence of TTX, this non-linearity in the current/voltage relationship is abolished. In voltage clamp experiments, mitral cells show voltage activation of a persistent inward current at these potentials. In the presence of TTX, the capacity for generating the up-state is lost; generation of the up-state in response to brief somatic depolarizations is abolished, and voltage oscillation at depolarized potentials no longer occurs. The data suggest that the mitral cell up-state represents a depolarized plateau potential generated, at least in part, by a persistent Na+ current, activated at potentials subthreshold for spike generation. This regenerative current may contribute to transition between two discrete levels of excitability in mitral cells. ON input may initiate depolarization to the up-state through activation of this regenerative current.

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163 Poster

DIFFERENTIAL EXPRESSION AND MODULATION OF AMPA AND KAINATE RECEPTORS IN MITRAL/TUFTED CELLS AND INTERNEURONS OF THE RAT OLFACTORY BULB

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The goals of this project were to determine which olfactory bulb (OB) neurons express which non-NMDA (AMPA/kainate) glutamate receptors and to characterize the biophysical and neuromodulatory properties of these receptors.

AMPA evoked AMPA receptor-mediated currents in the majority of mitral/tufted (M/T) cells and interneurons. However, M/T cells generated currents with properties typical of the flip-type splice variant, whereas flop-type receptors were predominant in interneurons. Our experimental results using cyclothiazide, which differentially potentiates flip versus flop splice variants, further support the notion of differential expression of AMPA receptor splice variants among OB neurons.

Kainate receptors also appear to be heterogeneously expressed. Kainate receptor-mediated currents were proportionally much larger in interneurons than M/T cells, suggesting a more significant role for kainate receptors in interneurons.

Zinc, a metal that is highly concentrated in the OB, had variable effects on AMPA or kainate receptor-mediated currents, including potentiation, inhibition, or no effect. The effects of zinc on AMPA receptors appear to be splice variant dependent and, therefore, suggest the possibility of cell-type-specific modulation. In contrast to zinc, copper blocked both AMPA and kainate receptors.

Our electrophysiological experiments, in which we altered extracellular calcium concentrations, and our immunocytochemical experiments, suggest most, but not all, OB neurons express the AMPA receptor subunit that prevents calcium permeability (GluR2). Furthermore, staining for kainate-receptor subunits GluR 5,6,7, and the AMPA-receptor subunit GluR2, showed that both receptor types may be presynaptically and postsynaptically localized on OB dendrites.

These results suggest: 1) M/T cells and interneurons express different types of AMPA receptors and different densities of kainate receptors, suggesting a mechanism for differential modulation of olfactory circuits; 2) AMPA and kainate receptors may mediate fast transmission postsynaptically but also modulate transmitter release presynaptically; 3) calcium-fluxing AMPA receptors may contribute to transmitter release at either presynaptic or postsynaptic sites at reciprocal synapses.

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164 Poster

DIFFERENTIAL EFFECTS OF ADAPTOR PROTEINS ON THE MODULATION OF AN OLFACTORY BULB ION CHANNEL BY V-SRC KINASE

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The interactions of protein tyrosine kinases with adaptor proteins direct communication between signal transduction components within the cell. We have shown previously that exogenous Src kinase suppresses outward current in voltage-clamped olfactory bulb neurons by phosphorylating the potassium channel, Kv1.3. We now show by Western analysis that c-Src and the adaptor proteins n-Shc and hGrb10α are expressed in the olfactory bulb. Phosphorylation and subsequent modulation of Kv1.3 by v-Src are found to be differentially regulated by adaptor proteins, as revealed by expression of the signaling components in HEK 293 cells. n-Shc and hGrb10α relieve the suppression of Kv1.3 peak current by v-Src by as much as 39.9% (n = 7) and 79.8% (n = 7), respectively. Additional modulation of Kv1.3 by v-Src is evoked by increasing deactivation kinetics and shifting V1/2 to more positive potentials. Both adaptor proteins relieve these modulated biophysical properties of Kv1.3, as well. n-Shc and hGrb10α differ in their effects on the phosphorylation state of Kv1.3 in the presence of v-src. Quantitative densitometric analysis shows that n-Shc increases phosphorylation of Kv1.3 by v-Src by 40.5% (n = 4), while hGrb10α decreases phosphorylation of Kv1.3 by v-Src by 88.3% (n = 3). Cummulative inactivation exhibited by Kv1.3 is modulated by n-Shc in the absence of v-Src, but the modulation of Kv1.3 current magnitude by n-Shc is phosphorylation-dependent, as demonstrated through use of a mutant Src and n-Shc cDNA constructs in which sites of kinase activity or phosphorylation of key tyrosine residues were altered, respectively. Our results show that the adaptor proteins n-Shc and hGrb10α regulate a modulator of ion channel activity by phosphorylation-dependent and -independent mechanisms. Protein-protein interaction motifs may direct modulation of ion channel activity in processing olfactory information in the bulb.

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165 Poster

ANALYSIS OF DOUBLE SPIKES IN MITRAL CELL PRIMARY DENDRITE

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The mitral cell of the rodent olfactory bulb gives rise to one primary dendrite entering a single glomerulus to receive olfactory nerve input. Our previous study has established that action potential traffic in this dendrite can be bi-directional, with either dendritic initiation and forward propagation or axo-somatic initiation and back-propagation (Chen et al. Science 278, 463-467, 1997). Here we have further analyzed the action potential-mediated communication between distal glomerular tuft and mitral cell soma with dual patch recordings in the slice. When the mitral cell soma is hyperpolarized either by current injection or inhibitory synaptic input to the secondary dendrites, the dendritic recording pipette often registered a spike doublet that corresponded to a single spike in the soma. The somatic spike was preceded by a fast prepotential and was later than the first spike of the doublet but earlier than the second one. We have constructed a mitral cell computational model to understand the membrane mechanisms underlying the double spikes. Even with a uniform distribution of sodium channels in the soma-dendrite membrane, simulation revealed that after initiation in the distal dendrite the first action potential jumped to the soma-axon region to trigger a full spike there, leaving a segment of proximal dendrite un-excited. This un-excited but excitable segment provides a basis for subsequent back-propagation of the somatic spike into the primary dendrite, yielding a second spike in the doublet. Our results thus indicate that significant spatial inhomogeneity in excitability can occur in long unbranched dendrites with uniformly distributed ion channels.

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166 Poster

THE PROPERTIES OF GRANULE CELL DENDRITIC SPINES IN CULTURE

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Dendritic spines are small appendages broadly distributed along the dendrites of many neurons. The dendritic spine is conventionally defined in terms of its role as the postsynaptic target of excitatory synapses. However, a notable exception is found in a population of anaxonic interneurons in the olfactory bulb. Granule cells are organized into local synaptic circuits with the major output neurons of the olfactory bulb, mitral and tufted cells. The granule cells have dendritic spines which both receive afferent synaptic inputs via glutamate receptors and make reciprocal efferent synaptic outputs via gamma-aminobutyric acid (GABA). The degree to which the bi-functional properties of the granule cell spine are induced by environmental conditions or are an expression of intrinsic determinants remain unknown. In order to establish whether isolated granule cell spines in vitro develop dual function components, we have employed high resolution confocal laser scanning microscopy on cultured granule cells fluorescently-labeled with phalloidin, which labels F-actin in spines, anti-synaptophysin to label synaptic vesicles, and anti-NR1 to label NMDA receptors. Staining with phalloidin clearly demonstrated the presence of spine-like appendages on granule cells in vitro, as did our parallel analyses with electron microscopy. Punctate NR1 labeling occurred along dendritic shafts, but did not appear to extend into the spine heads. Synaptophysin staining was also present indicating that the components of the dual function spines are present in granule cells in vitro, but that they have not yet migrated into the spine head. Presently, we are exploring determinants that may influence the final targeting of both the NMDA receptors and synaptic vesicles into the head of the dendritic spine.

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167 Poster

TIME-DEPENDENT NEUROMODULATION OF OLFACTORY BULB NEURON CURRENT BY RECEPTOR-LINKED TYROSINE KINASES AND RELATED GROWTH FACTORS

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Kv1.3, a voltage-gated potassium channel predominantly found in the olfactory bulb (OB), has previously been shown to be modulated by receptor-linked tyrosine kinases (RTKs) in a heterologous expression system. We now show several RTKs (Trk A, Trk B, Trk C, and IR) are present in OB membrane preparations by Western analysis. Patch- clamped rat OB neurons acutely stimulated for 15 minutes with 50 ng/ml of bath applied NT3 (n = 6), BDNF (n = 7), NGF (n = 4), insulin (n = 7), IGF-I (n = 8), or IGF-II (n = 2), show a 19 ± 8% suppression of outward current with BDNF and a 24 ± 6% suppression with insulin. Other biophysical properties such as $V_{1/2}$, inactivation kinetics, and deactivation kinetics were not significantly affected by acute stimulation. Tyrosine phosphorylation of Kv1.3 increased two fold when OBs were acutely stimulated with BDNF as demonstrated by Western analysis and quantitative densitometry. Insulin-induced tyrosine phosphorylation of the channel was time-dependent and rapid, demonstrating increases after only 30 seconds of stimulation. OB homogenates contained a moderate level of insulin compared with plasma as detected by ELISA. Following a 72 hour fast, insulin levels increased four fold, suggesting retention in the brain. When OB neurons are chronically stimulated (24-216 hrs) with the same battery of RTK ligands, we find incremental increases in peak current amplitude through DIV above that of time-matched controls (n = 50) for NT3 (n = 50) and BDNF (n = 44). As found with the acute trials, other biophysical properties were not affected. We suggest two putative mechanisms modulate the peak amplitude of outward currents in OBNs: (1) tyrosine phosphorylation of Kv1.3 channels by IR kinase or Trk B during rapid stimulation of neurons by modulators and (2) decreased phosphorylation of channels during constant internalization of the RTKs.

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168 Poster

FUNCTIONAL DEVELOPMENT OF CONNECTIVITY BETWEEN THE VNO AND AOB

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The answer to the question of the onset of function in the mammalian accessory olfactory system remains unresolved. If this system is functional in the newborn and young, it would indicate that the vomeronasal receptor neurons (VRNs) in the nasally-located vomeronasal organ (VNO) are capable of transmitting the pheromonal information to the accessory olfactory bulb (AOB) and that the AOB is capable of responding and transmitting that information to other parts of the brain. A role for this system during early development also might be particularly significant for the human species, where a VNO and an AOB have been demonstrated in fetuses. The present study is using immuno-labeling for calretinin (CR), calbindin (CB), and protein gene product 9.5 (PGP) of VNO receptor neurons (VRNs) and AOB target neurons at various stages of fetal development in a rat model to determine the onset of expression of these functionally-important calcium-binding proteins (CR and CB) and compare their initial time of expression with that of PGP, believed to be involved in neuronal differentiation. Retrograde tract-tracing with DiI applied to the VNO also is being employed to establish the initial contact of VRNs with their AOB targets at the same stages of development. CR and PGP are both expressed in VRNs as early as embryonic day 17. Immunoreactivity for both proteins was detected throughout the cytoplasm and into the axons of a set of VRNs. CR and PGP expression also has been detected in AOB neurons at this age. CR and PGP expression at younger ages, along with CB-immunolabeling studies are in progress. Tract-tracing has shown DiI-labeled fibers projecting from late-stage embryonic VRNs toward the AOB. Studies with earlier-stage embryos are continuing to compare the onset of expression of functionally-important proteins with physical contact between VRNs and their target neurons in the AOB.

169 Poster

NORADRENERGIC MODULATION OF DENDRODENDRITIC SYNAPTIC TRANSMISSION IN THE RAT ACCESSORY OLFACTORY BULB

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Mitral/tufted (M/T) cells of the accessory olfactory bulb (AOB) form dendrodendritic synapses with granule cells and other inhibitory interneurons. Modulation of the dendrodendritic synapses is believed to underlie some forms of olfactory learning. Olfactory learning also depends on activation of the central adrenergic system (Brennan et al., 1990). There is no data, however, on how the central adrenergic system modulates dendrodendritic transmission in the AOB. In the present study, the effects of norepinephrine on dendrodendritic synaptic transmission in the AOB were analyzed in slice preparations using field potential and whole-cell patch recordings. Norepinephrine (20 microM) in the bath medium increased EPSPs in granule cells evoked by M/T cell stimulation. Norepinephrine also increased feedback IPSPs in M/T cells if the IPSPs were suppressed by metabotropic glutamate receptor agonist DCG-IV. In addition, application of norepinephrine in the bath depolarized M/T cells and could induce firing. The same method applied to the main olfactory bulb had limited effect. These results indicate that the role of the adrenergic system in the AOB may be different from that in the main olfactory bulb. Further study is therefore needed to test whether olfactory learning in the main and the accessory olfactory bulb may have different machanisms.

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170 Poster

SENSITIZATION, DESENSITIZATION AND STIMULUS-INDUCED RECOVERY OF RESPONSES OF RAT TRIGEMINAL CAUDALIS (VC) NEURONS TO REPEATED ORAL APPLICATION OF CAPSAICIN

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Repeated application of capsaicin to the oral mucosa produces a progressive increase in perceived irritation (sensitization), followed after a rest period by reduced sensitivity to capsaicin (desensitization). With recurrent application of capsaicin, irritation increases again to attain the initial level, a phenomenon called 'Stimulus Induced Recovery' (SIR). We investigated if neurons in trigeminal subnucleus caudalis (Vc), which are thought to signal oral chemical irritation, show response patterns that match human sensation. In thiopental-anesthetized rats, single-unit recordings were made from superficial laminae of Vc. We sought wide dynamic range (N = 17) or nociceptive-specific (N = 12) units that responded to noxious thermal (54°C), mechanical, and chemical (pentanoic acid; capsaicin) stimuli. A series of 25 capsaicin stimuli (0.1 ml, 330 μ M) were repeatedly applied at a 1-min interstimulus interval to the tongue. Responses of 11 Vc units increased significantly over the first 8-10 trials to a plateau that was maintained throughout the stimulus series. After a >30 min rest period, firing had returned to the pre-capsaicin level, and the identical series of capsaicin stimuli was reapplied. The units' response again increased, but only after a significant delay consistent with desensitization. The maximal firing rate that was significantly lower compared to the initial stimulus series, indicative of a partial SIR. Virtually identical results were obtained in separate units (N = 7) receiving a continuous flow of capsaicin (0.32 ml/min) for 25 min, and again >30 min later. In contrast, a single application of capsaicin induced a significantly smaller and slower increase in activity in 8 other units, suggesting that sensitization requires a constantly maintained capsaicin concentration, and there was no evidence for SIR following a second singular capsaicin stimulus. These results are consistent with the phenomena of sensitization, desensitization, and SIR observed in humans, except that the SIR of Vc units was only partial.

171 Poster

EXPRESSION PATTERN OF THE PROTEIN CODED BY THE IMMEDIATE-EARLY GENE *ARC* IN THE ACCESSORY OLFACTORY BULB AFTER EXPOSURE TO PHEROMONAL STIMULI.

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The accessory olfactory bulb (AOB), which has five layers; the vomeronasal nerve layer, the glomerular layer (GL), the mitral/tufted cell layer (MTL), the olfactory tract layer and the granule cell layer (GRL), is the first relay station in the vomeronasal system. Recent studies on the AOB have shown that the expression of immediate-early genes, e.g., *c-fos*, *c-jun* and *egr-1*, can be used as a marker of neuronal activity in response to pheromonal cues. In this study, we analyzed the expression pattern in response to pheromonal stimulation of the protein product (Arc: activity-regulated cytoskeleton-associated protein) of the novel immediate-early gene *Arc*, which is hypothesized to play a role in activity-dependent neuronal plasticity in the hippocampus. We adopted as the pheromonal stimuli, exposure of the adult male rat to the soiled bedding of female rats, or contact with female rat. In the control group, a few Arc immunoreactive (Arc-ir) cells were observed throughout all the layers of the male rat AOB. In the group allowed to come in contact with the female, a marked increase in the number of Arc-ir cells was confirmed in the GRL. In the group exposed to the soiled bedding of females, an increase in the number of Arc-ir cells was observed in the GRL, but the increase was smaller than that in the female contact group. A few Arc-ir cells were observed in the GL and MTL of both stimulated groups. Thus, the number of Arc-ir cells was increased after pheromonal stimulation as clearly as the case for that of the other immediate-early genes, but the increase was localized only in the GRL. It has been reported that the granule cells exhibit strong synaptic plasticity in response to pheromonal stimulation. It is thus possible that Arc plays an important role in neuronal plasticity in AOB.

172 Poster

FEMALE-SOILED BEDDING C-FOS IMMUNOREACTIVITY IN THE VENTRAL PART OF THE PREMAMMILLARY NUCLEUS (PMV) OF THE MALE MOUSE

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Previous studies have indicated that the ventral part of the premammillary nucleus (PMv) of rodents is involved in the regulation of male mating behavior and pheromone inducible LH release, although the precise physiological function of the PMv is still unclear. To analyze the physiological role of the PMv in LH release and/or mating behavior, the effects of exposure to bedding soiled by female mice on c-Fos immunoreactivity (Fos-ir), an early marker of neuronal activation, were studied in the PMv, the accessory olfactory bulb (AOB) and some sex-related nuclei. We observed that exposure to female-soiled bedding induced Fos-ir expression in the PMv and AOB of the male mouse. Although Fos-ir positive cells were found in the anterior- and posterodorsal part of the medial amygdaloid nucleus and in the posterior nucleus amygdala, which are terminals of the neuronal projections from the accessory olfactory bulbs, the numbers of Fos-ir cells in those nuclei were not affected by exposure to female-soiled bedding. Moreover, Fos-ir was not detected in the ventromedial hypothalamic nucleus. It is well established that soiled bedding is useful as a source of chemosensory substances, which include "pheromones". Thus our findings, in agreement with previous behavioral and anatomical data, suggest that the male PMv play a role in initiating male copulative behavior and/or LH release that is induced by a female pheromone(s).

173 Poster

FOS EXPRESSION IN MEDIAL PREOPTIC AREA DUE TO INTRACEREBRAL LHRH INJECTION IN INTACT MALE HAMSTERS AND THOSE WITH VOMERONASAL LESIONS BEFORE OR AFTER EXPERIENCE.

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Intracerebroventricular (icv) injections of LHRH substantially restore mating behavior in male hamsters vomeronasal lesions (VNX) (Fernandez-Fewell and Meredith 1995). Immunocytochemical studies show significantly more cells in mid-caudal medial preoptic area (MPOA) with Fos activation in LHRH injected-males compared to saline-injected males during mating; or after exposure to hamster vaginal fluid (HVF, a source of mating pheromones) without mating. Thus, the combination of chemosensory and hormonal inputs appeared to activate cells in a region of brain known to be involved in the initiation of mating. Inexperienced VNX males,whether mating or HVF-exposed, showed no detectable increment of Fos expression in the MPOA attributable to the exogenous LHRH (Westberry and Meredith 1999). Possibly, cells were activated by the conjunction of chemosensory (olfactory) and hormonal inputs; but at insufficiently high levels for detectable Fos expression. In mid-caudal MPOA, Fos expression due to exposure to HVF is higher in experienced than in inexperienced VNX males suggesting enhanced activation by chemosensory input after experience. We repeated the LHRH-injection experiment in experienced VNX animals to see if enhanced chemosensory input would allow LHRH to increase Fos expression. However, neither experienced nor inexperienced males showed any additional Fos activation in MPOA attributable to exogenous LHRH. Testosterone and LH increases following chemosensory stimulation disappear in VNX males, whether experienced or not (Pfeiffer and Johnston 1994), implying that there is no endogenous LHRH response in VNX males. Thus, the increment in Fos positive cells in MPOA may reflect a combination of exogenous and endogenous LHRH rather than a combination of hormonal and chemosensory input. Alternatively, VNO input may be essential for activation of these MPOA cells by LHRH, the behavioral facilitation in VNX males being dependent on convergence elsewhere.

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174 Slide

IDENTIFICATION OF MESSENGER RNAS ENRICHED IN THE LOBSTER OLFACTORY ORGAN

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We have detected 27 cDNA fragments representing mRNA species that are enriched in the olfactory organ. Representational Difference Analysis (RDA) of cDNA was used to amplify cDNA fragments enriched in the olfactory organ cDNA compared to a mixture of brain and second (large) antennae cDNA. The cDNA fragments were obtained in the second difference product which typically yields species that are enriched at least two-fold in the target DNA pool. These products were cloned into pBluescript, analyzed for insert size, and sequenced. Cross hybridization of pBluescript transformed bacterial colonies was used to confirm that all species in the difference product had been detected. Six of the difference product clones had significant similarity to : (1) ionotropic glutamate receptors (2 clones), (2) dopamine β-hydroxylase (3 clones), (3) a tubulin, (4) a calcium binding protein (2 clones), (5) trypsin/chymotrypsin, and (6) an a 2-macroglobulin (2 clones). The remaining 17 sequences had no significant similarity to known sequences. We confirmed that all our difference products were enriched in the olfactory organ by RNA dot blot, cDNA dot blot, and Northern blots. This extremely high fidelity is typical of RDA procedures that we, and others, have performed previously. Experiments to determine which cell types in the olfactory organ express particular difference product clones are in progress.

Surprisingly, no fragments were amplified in the third difference product, which typically contains products that are many-fold enriched in the target tissue DNA. We have subsequently reproduced this result. Given the fidelity of RDA, this result suggests the hypothesis that the lobster olfactory organ contains few, if any, mRNA species that are not also expressed in the brain or second antennae. This is consistent with evidence that chemoreceptors elsewhere on the animal, including the second antenna, share molecular mechanisms with olfactory organ chemoreceptors.

175 Slide

CIRCADIAN CONTROL OF OBP TRANSCRIPT LEVELS IN *DROSOPHILA MELANOGASTER*

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We have previously shown that olfactory responses in Drosophila are under circadian control1. Briefly, electroantennogram (EAG) responses to multiple odorants vary over the course of the day, with a peak in the middle of the night. This pattern persists in constant-dark conditions, and is abolished in flies bearing null-mutations in circadian clock genes. Studies with transgenic flies suggest that this rhythm is controlled by circadian oscillators located in the periphery. Because OBPs are secreted molecules, they provide a reasonable substrate for circadian control of olfactory responses. We now report that at least one OBP transcript, as determined by RNAse protection assays, is regulated by circadian clocks. Transcript levels exhibit a moderate peak early in the subjective night, shortly before peaks in physiological responses. The rhythm persists in constant-dark conditions and preliminary data suggest that it is abolished in period null-mutant flies. Interestingly, at least one other OBP transcript is not rhythmic in light-dark cycles or constant darkness. Circadian rhythms in olfactory responses may provide a mechanism to ensure robust olfactory responses in the face of changes in odorant vapor pressure associated with daily fluctuations in ambient temperature. Alternatively, they may provide a mechanism to optimize foraging, avoid predators, or regulate specific behaviors that require temporal organization. Ongoing experiments are designed to investigate these rhythms at the protein level.

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176 Slide

SENSORY NEURON MEMBRANE PROTEIN (SNMP) DIVERSITY IN THE SPHINX MOTH *MANDUCA SEXTA*

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SNMP is an antennal specific receptor protein uniquely localized in the receptor membranes of olfactory receptor neurons (ORN). A current hypothesis is that SNMP coordinates the off-loading of pheromone molecules from Pheromone Binding Proteins (PBP), allowing pheromone to be delivered to neighboring transductory receptor proteins. SNMP was first isolated from the ciliary membranes of sex-pheromone specific ORNs of the wild silk moth *Antheraea polyphemus*. SNMP1-Apol was thought to play a receptor-like role in odor detection based on its olfactory-specific expression, ORN localization, and apparent homology to the CD36 family of membrane-bound receptor proteins. The specific receptor-like roles ascribed to the CD36 proteins, along with recent biochemical evidence suggesting that pheromone release from the pheromone-PBP complex may require an interaction with proteins in the ORN membrane, supports a role for SNMP1-Apol as a docking receptor for the pheromone-PBP complex. In the present study, SNMP1-Apol homologues were isolated from the moths *Manduca sexta, Bombyx mori* and *Heliothis virescens*. These sequences, along with a second independently identified *M. sexta* SNMP (SNMP2-Msex), represent an emerging family of novel olfactory proteins defined by their unique expression in the ciliary membranes of ORNs. Amino acid identities among the SNMP1 proteins range from 67-73%, while identities between SNMP2-Msex and the SNMP1 proteins are about 40%. Both SNMP1-Msex and SNMP2-Msex are antennal specific and express in olfactory neurons based on Northern blot analysis and in situ hybridization studies. Developmental Northern blot analysis indicates a low level of SNMP1-Msex expression beginning around 85% of adult development and increasing dramatically by 94% of development, coincident with the functional maturation of the olfactory system. The identification of SNMP homologues in multiple insect species suggests that the SNMPs are a general feature of olfactory neurons, and the expression studies suggest the SNMPs have a central role in odor reception.

177 Slide

IMMUNOLOCALIZATION OF FIVE ODORANT-BINDING PROTEINS ON THE ANTENNAE OF *DROSOPHILA MELANOGASTER*

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Using molecular cloning a great number of putative odorant-binding proteins (OBPs) have been characterized in *Drosophila melanogaster* (1, 2, 3). Recombinant proteins were used to raise polyclonal antibodies against the following OBPs: OS-E, OS-F, PBPRP2, PBPRP5, LUSH. In a post-embedding labelling protocol we used these antisera on ultrathin sections of cryofixed *Drosophila* antennae in order to find out the localization of these OBPs at electron microscopic resolution.

The resulting expression pattern in the different types of olfactory sensilla was complex but persistent. OS-E, OS-F and LUSH were always co-localized and were present in the great majority of sensilla trichodea. Thus, s. trichodea express three different OBPs in the same sensory hairs. OS-E and OS-F, but not LUSH were also labelled in the s. intermedia, a type that combines features of s. trichodea and s. basiconica (4, 5). PBPRP5 was observed in a subset of large s. basiconica, while PBPRP2 was expressed in a very small fraction of the s. coeloconica on the antennal surface and, surprisingly, in the subcuticular space between ordinary epidermal cells and antennal cuticle (6). Thus, the different OBPs are found distributed in a type-specific pattern on the antenna of *Drosophila*. Experiments to elucidate their function are underway.

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178 Slide

RESPONSE OF *DROSOPHILA* MALES TO FEMALE PHEROMONES INVOLVES SPECIALIZED CHEMOSENSORY HAIRS ON THE MALE FRONT LEGS.

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Much is known about the complex courtship behavior of _*Drosophila* as well as the chemistry of activating and inhibiting pheromones. In contrast, the chemosensory organs, cells and molecules involved are largely unknown. We have cloned two *Drosophila* genes whose remarkably specific expression patterns suggest a role in the perception by males of courtship-modulating pheromones, *Grenouille1(Grl1)* and *Bergerac1 (Bgc1)*. Both genes are only expressed in a few cells at the base of chemosensory hairs in the front legs of males, an organ previously implicated in perception of female pheromones. In addition, the cells expressing BGC1 are only present in a small subset of chemosensory hairs that do not express PBPRP2, a protein found in many chemosensory organs, including most leg chemosensory hairs. Interestingly, while neither removal of antennae nor specific ablation of *Bgc1*-expressing cells affects male perception of female pheromones, performance of both operations on the same males results in complete loss of response. Our data suggest that males can use either of two redundant chemosensory pathways to detect female courtship-stimulating pheromones, one involves the antennae, the other requires specialized *Bgc1*-expressing cells in the front legs.

179 Slide

MOLECULAR GENETICS OF OLFACTION IN THE MALARIA VECTOR MOSQUITO ANOPHELES GAMBIAE

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The ability to sense and discriminate a large collection of chemical and visual cues is central for several behaviors of insects that are agricultural pests or vectors for the pathogens responsible for many important human diseases. In particular, olfaction plays a major role in host seeking and selection behaviors of blood feeding female mosquitoes and as such, constitutes a critical component of the mosquito's ability to transmit diseases such as malaria, encephalitis and dengue. In as much as an increased understanding of these chemosensory mechanisms may be useful in the development of novel control strategies a molecular characterization of olfaction within mosquitoes of the Anopheles gambiae (senso lato) species complex has been undertaken. This group of mosquitoes includes non-vector species as well as the principal Afrotropical malaria vector species An. gambiae (senso stricto) whose strong preference for human hosts (anthropophily) is largely responsible for its high vectorial capacity.

The long term objectives of our research is centered on an examination of the molecular genetics of olfaction and its role in determining anthropophilic host preference. Data will be discussed concerning the characterization of previously identified representatives of two families of genes that make up essential elements of the peripheral olfactory signal transduction cascade in An. gambiae s.s. These encode Arrestins and Odorant Binding Proteins (OBPs), which together with their corresponding Odorant Receptors represent the peripheral components for signal transduction associated with olfactory chemo-sensation. We have examined arrestin and OBP localization within the mosquito's olfactory apparatus. Furthermore, data will be presented to suggest that the well established circadian rhythms of host seeking behaviors influence the specific temporal expression patterns of olfactory genes such as arrestin.

184 Poster

DYNAMICS OF OLFACTORY RECEPTOR NEURON TURNOVER IN THE SPINY LOBSTER

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A developmental gradient of olfactory receptor neurons (ORNs) exists in the antennule of the spiny lobster *Panulirus argus*, with ORNs and their associated aesthetascs added proximally and shed distally. We have examined the dynamics of the cellular events involved in establishing and maintaining this developmental gradient, using markers for cell proliferation (BrdU), maturation (activity labeling and intracellular taurine), and death (TUNEL). BrdU labeling shows that there is a 'primary' wave of ORN proliferation that travels continuously in the proximal direction, which results in the formation of ORN clusters prior to the formation of their associated sensilla. The rate of ORN proliferation is dependent on molt stage: proliferation rate increases shortly before molt and remains elevated for several days after molt. During this time, ORN clusters are added lateral to the existing, newly formed clusters. We are investigating the possibility that this lateral addition is the result of a molt-cycle-dependent 'secondary' wave that travels from the mesial to lateral margin of the antennule. Activity labeling studies have shown that newly formed ORNs require at least three weeks to differentiate into mature, odor-responsive neurons. BrdU labeling did not reveal proliferation among mature clusters of ORNs, but the TUNEL technique revealed apoptosis, particularly among clusters in more distal regions. Thus in the lobster, turnover occurs through the continuous addition of ORN clusters in the proximal portion of the antennule, and the removal of old clusters through cell death and shedding in the distal portion. Individual ORNs do not appear to be replaced within established, mature ORN clusters. Thus, the lobster olfactory system is similar to many olfactory systems in that ORNs undergo continuous turnover in adults, but differs by having the areas of proliferation and death largely segregated from each other.

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185 Poster

TEMPORAL PROFILE OF BAX AND BCL-2 GENE EXPRESSION FOLLOWING BILATERAL BULBECTOMY IN THE RAT: A MODEL FOR EXAMINING THE MOLECULAR REGULATION OF NERUONAL APOPTOSIS.

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The olfactory epithelium (OE) of the rat provides a unique system for understanding the molecular regulation of neuronal apoptosis. The pro-apoptotic *bax* gene and the protective *bcl-2* gene encode proteins which regulate programmed cell death. The ratio of these proteins helps determine whether cells will undergo apoptosis. The standard histologic marker for this process is the terminal deoxynucleotidyl-transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) assay, which reveals only a small number of apoptotic neurons in the normal OE. A dramatic increase in the number of TUNEL positive cells in the OE, however, can be seen following bilateral bulbectomy. To elucidate the process of gene activation following injury-induced apoptosis in this system, we investigated the temporal profile of *bax* and *bcl-2* expression. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and slot blot analysis revealed that *bax* gene expression was up-regulated as early as one day post-bulbectomy, continued to increase to 1.5-2 fold its baseline value at 2 days post-bulbectomy, and peaked at 9 days post-bulbectomy to 20 fold its baseline value. *Bax* gene expression returned to baseline normal values at 1 month post-bulbectomy. Parallel immunohistologic studies also detected increased immunoreactivity for Bax protein in the OE 48 hours after bilateral bulbectomy with peak amount of staining occurring 9 days after bulbectomy. Expression of *bcl-2* mRNA and Bcl-2 protein, however, showed no obvious changes at any time following the injury. These results suggest that olfactory neuronal apoptosis following bulbectomy is associated with increases in the level of Bax protein expression.

186 Poster

CELL TURNOVER IN THE VOMERONASAL EPITHELIUM: EVIDENCE FOR DIFFERENTIAL MIGRATION AND MATURATION OF SUBCLASSES OF VOMERONASAL NEURONS IN THE ADULT OPOSSUM.

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Previous investigations of cell turnover in the mammalian vomeronasal sensory epithelium (VN-SE) raised two issues. First, is the migration of newly generated neurons vertical and/or horizontal' Second, since the apical and basal receptor cell populations are chemically, physiologically, functionally and, perhaps, evolutionarily different, is the rate of migration and maturation different for these two neuronal populations? We injected bromodeoxyuridine (BrdU) into adult opossum (Monodelphis domestica), permitted different survival times and analyzed the pattern of distribution of BrdU-labeled cells. As previously reported by Jia and Halpern (1998, J Comp Neurol 400: 287-297) no evidence of horizontal migration in neuronal replacement was found and there was substantial evidence for vertical migration from basal to apical regions of the VN-SE. To investigate vertical migration and maturation of subclasses of vomeronasal neurons, double immunohistochemistry of BrdU and markers of the basal (Goα protein) and apical (Gi2α protein and olfactory marker protein, OMP) cell populations were performed. Three days after administration of BrdU, some mature neurons were observed in both, basal and apical layers of the VN-SE, as demonstrated by co-expression of BrdU with Goα protein and OMP, respectively. The data on vertical distribution indicate that most of the daughter cells enter the Goα -protein-expressing zone of the VN-SE by day 5, whereas most daughter cells do not reach the Gi2α -protein-expressing zone until day 7, suggesting that these two populations mature at slightly different rates. These results are the first evidence of differential neurogenesis of subclasses of vomeronasal neurons.

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187 Poster

AGING ALTERS GENE EXPRESSION PROFILES IN THE RAT OLFACTORY MUCOSA

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Aging is a natural condition that exerts stress on many homeostatic functions throughout the organism. These homeostatic functions are in turn controlled by interconnected biological pathways, ultimately regulated by gene expression. Control of homeostasis can therefore be perturbed by persistent changes in gene expression. We set out to determine if there are changes in the gene expression profile of the rat olfactory mucosa that are associated with aging. To this end, gene expression profiles were constructed using RNA purified from the olfactory mucosa of rats from different age groups. Profiles were generated by hybridization of radiolabelled reverse transcribed mRNAs to gene array membranes. The resulting mRNA profiles allowed simultaneous comparison among animals of different age groups of hundreds of genes expressed in the olfactory mucosa. To aid comparison, genes were organized on arrays according to known functional categories such as stress response, transcription factors, cell-cell communication and apoptosis. Many genes such as GAP 43, PDGF-B and Clusterin/ApoJ were identified as either up or down regulated in older animals as compared to younger animals. Several of these genes have already been implicated as relevant to olfactory function. These genes were further evaluated by immunohistochemistry to confirm that changes in mRNA levels resulted in a corresponding change in respective proteins. This work demonstrates that factors relevant to aging of olfactory neurons can be identified by application of panaoramic gene expression analysis using gene array technologies.

188 Poster

APOLIPOPROTEIN E PEPTIDE INCREASES INTERNAL CALCIUM IN MATURE OLFACTORY RECEPTOR NEURONS TAKEN FROM ADULT RATS

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Apolipoprotein E (apoE) may be involved in neurite outgrowth, degeneration and regeneration. It has also been associated with neurotoxicity and Alzheimer's disease. In nondemented elderly humans, apoE immunoreactivity is found in the ensheathing cells that surround the axons of the olfactory receptor neurons (ORNs), along the epithelial basement membrane, and in some ORNs (Ann. Otol. Rhinol. Laryngol. 107:421 (1998)). In Alzheimer's disease, the number of apoE immunoreactive ORNs increases (ibid). In the few neuronal populations that have been tested, an apoE peptide and a truncated form of apoE (both containing apoE's receptor-binding domain) increased internal calcium (e.g., J. Neurosci. 19:7100 (1999)). Because the ensheathing cells may be an apoE source that would affect ORNs, we monitored calcium in mature ORNs from adult rats to see if they were capable of responding to the synthetic tandem apoE peptide $E_{(141\text{-}149)}2$, a duplicated sequence of the receptor-binding domain of apoE (amino acids 141 through 149) that is the same in all three known human apoE isoforms.

Olfactory epithelia from adult rats were disaggregated and the cells were plated on concanavalin A-coated coverslips. The next day, the cells were loaded with the calcium indicator dye, Fluo-4. A subpopulation of the mature ORNs responded to the apoE peptide (6 μ M) with an increase in internal calcium. The mature ORNs were identified by relocation after immunocytochemistry for olfactory marker protein (antibody provided by Dr. F. Margolis). We plan to use this model to explore the poorly understood mechanisms of apoE transduction.

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189 Poster

AN IN VITRO SYSTEM TO STUDY AFFERENT INFLUENCE ON TARGET NEUROGENESIS

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The olfactory receptor neuron axons have been demonstrated to influence the formation of their synaptic target in the brain, the olfactory bulb. However, many questions remain about the molecular mechanisms that regulate cytogenesis, survival and maturation of specific cell types in the olfactory bulb. Previous studies have suggested that the pioneer olfactory axons are involved in regulating the formation of the bulb. We have established an explant culture system that will allow detailed cellular and molecular analysis of this process. We harvested E11 mouse embryos and cultured the presumptive olfactory bulb, with and without attached olfactory epithelium, in a collagen matrix with defined medium. Cell cycle parameters in the presumptive bulb explants were analyzed with a cumulative S phase labeling method, using bromo-deoxyuridine as a marker of cells passing through S phase. After 20 hrs in culture, the cell cycle progression in olfactory bulb explants with olfactory epithelium attached displayed similar parameters to those of in vivo specimens, with a comparable cell cycle duration of 12.5 hrs and an S phase duration of 6 hrs. However, the growth fraction was substantially lower, 0.55 compared with 1.0 in vivo. Assays of cell cycle parameters in olfactory bulb explants without olfactory epithelium are currently underway to determine the role of olfactory axons on the cell cycle progression in the presumptive olfactory bulb. This in vitro system will provide an entry point for further molecular analysis.

190 Poster

SEMAPHORIN 3A IS REQUIRED FOR NORMAL GUIDANCE OF OLFACTORY AXONS IN MICE.

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Semaphorin 3A is a membrane-associated secreted protein that has chemorepulsive properties for neuropilin-1 expressing axons. Mice lacking the Sema3A protein have abnormal bone and cartilage structures and the right atrium and ventricle of the heart are malformed. While mice lacking the Sema3A protein display skeletal abnormalities and heart defects, most axonal projections in the CNS, surprisingly, develop normally. Semaphorin 3A is expressed in the lamina propria in the nasal cavity and by ensheathing cells in the nerve layer of the ventral olfactory bulb beginning at E12 and continuing throughout development. Subsets of sensory neurons expressing neuropilin-1 are distributed throughout the OE and extend fibers to the developing OB. In wild-type mice, neuropilin-1+ axons extend to medial and lateral targets, avoiding the ventral midline of the OB where Sema3A is preferentially expressed. In Sema3A homozygous mutant mice, many neuropilin-1+ axons are mis-routed into ventral and dorsal targets, beginning as early as E13 and continuing at least until birth. In addition, subsets of OCAM+ axons that normally project to the ventrolateral OB, and some LCG+ axons that normally target the ventral OB, are also mis-routed in Sema3A mutants. At postnatal day 0, the formation and positions of glomeruli are also aberrant in Sema3A mutant mice. There are additional neuropilin-1+ glomeruli in the ventral OB of mutant mice and there are many fewer and smaller OCAM+ glomeruli in Sema3A mutants compared to wild-type littermates. These observations indicate that Sema3A expression by ensheathing cells at the ventral midline of the OB is essential for initial patterning of sensory projections to the olfactory bulb.

191 Poster

DIFFERENTIAL EXPRESSION OF GAL-NCAM, A NEW NCAM GLYCOFORM IN THE RAT OLFACTORY SYSTEM

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In this study we describe 2E11, a monoclonal antibody which is specific for a blood group B epitope present both on a glycolipid and on Gal-NCAM, a new NCAM glycoform. In embryos, Gal-NCAM has a restricted pattern of expression. In the main olfactory system, only a subset of sensory olfactory neurons expresses Gal-NCAM. These neurons can be found in all four of the receptor-defined zones of the olfactory epithelium whereas their axons converge mainly on the medial nerve layer of the olfactory bulb. In the accessory olfactory system, sensory neurons expressing Gal-NCAM are located basally in the vomeronasal epithelium and project axons into the caudal glomerular layer of the accessory olfactory bulb. Additionally, Gal-NCAM can be considered as a marker for mature neurons. Indeed, comparative studies between Gal-NCAM and PSA-NCAM show that these two antigens have a mutually exclusive pattern of expression. Furthermore, most Gal-NCAM immunopositive neurons also express OMP (Olfactory Marker Protein), a maturation marker for olfactory neurons. Finally, using neuraminidase treatment on paraffin sections, we show that the blood group B epitope can be masked by sialic acid residue(s).

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192 Poster

EXPRESSION OF THE INTERMEDIATE FILAMENT PROTEIN, NESTIN, IN THE MATURE OLFACTORY NEUROEPITHELIUM

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The intermediate filament protein, nestin, has been widely used as a marker for proliferating progenitor cells in the developing nervous system. The mammalian olfactory neuroepithelium, which supports ongoing neurogenesis, has been somewhat exceptional in being reported negative for expression of nestin by its proliferating neuronal progenitors (Dalstrand et al, 1995). These olfactory progenitors reside in the globose basal cell layer at the base of the neuroepithelium and give rise to daughter cells which move apically during neuronal differentiation. Using immunohistochemistry, we examined nestin expression in the mature olfactory neuroepithelium and found it to be restricted to the basal compartment of the neuroepithelium. The pattern of immunoreactivity was consistent with expression of nestin by the endfeet and inferior processes of the sustentacular cells, rather than the adjacent basal cells. Using a bank of antibody markers, we confirmed nestin's pattern of distribution to be different to that of cytokeratin, the GBC-1 antigen used to mark globose basal cells, GAP43, carnosine and vimentin. Following unilateral surgical bulbectomy, nestin immunoreactivity was upregulated bilaterally and appeared to span the neuroepithelium from apical to basal regions, also becoming prominent in the cell bodies of some sustentacular cells. We have shown nestin to be present in the basal region of the adult neuroepithelium, in the zone containing olfactory stem cells and neuronal precursor cells, where it was most avidly expressed by sustentacular cell endfeet. Nestin may play a role in the migration of recently proliferated olfactory neurons on the scaffolding of sustentacular cells, in a manner analogous to its proposed role in radial glial cells during embryonic development of the central nervous system. The upregulation in sustentacular cells postbulbectomy may reflect the intense requirement for cell mobility and remodelling in the regenerating neuroepithelium.

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193 Poster

EFFECT OF VITAMIN A ON THE MRNA EXPRESSION LEVELS OF OLFACTORY MARKER PROTEIN

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Dietary vitamin A (VA) is the only known source of endogenous retinoic acid (RA), amolecule known to affect gene expression and cell differentiation. We are interested in determining whether VA affects the regeneration and differentiation of olfactory neurons in mature rats. Our working hypothesis is that dietary deprivation of VA will compromise neurogenesis if RA is required in the process. Since olfactory marker protein (OMP) is expressed in mature neurons, but not immature neurons of the olfactory epithelium, we used it to track the effect of VA deficiency on the maturation of olfactory sensory neurons. Total RNA was isolated from the olfactory mucosa of VA-sufficient (VAS) and VA-deficient (VAD) rats. The levels of OMP mRNA were analyzed in these tissues by reverse transcriptase polymerase chain reaction (RT-PCR). The RT-PCR product was cloned and sequenced to verify its authenticity. To accurately and reproducibly quantify gene expression levels, a protocol based on QuantumRNA™ (Ambion) technology was developed and evaluated using 18S RNA and β-actin as internal standards. Bio-RAD Quantity One™ software was used for densitometric analysis. Based on this analysis, we have determined that OMP mRNA expression levels in VAD rats are reduced to 40% of control. These data are consistent with the notion that availability of VA affects neurogenesis in the mature rat OE.

194 Poster

EXPRESSION OF CELLULAR RETINOIC ACID BINDING PROTEINS IN MATURE RAT OLFACTORY EPITHELIUM

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Indirect evidence obtained from embryonic studies and cultured cells indicates that retinoic acid (RA), an oxidized derivative of vitamin A (VA), may be required for cells to commit to a neuronal phenotype. Since neurons are regenerated throughout adult life in the olfactory epithelium (OE), one would expect to find markers of RA metabolism present in OE if RA does play an essential role in neurogenesis in mature animals. Consistent with this expectation, we have observed expression of the cellular retinoic acid binding proteins, CRABP type I and CRABP type II in mature rat OE. Reactivity with antibodies to these proteins is observed in the supranuclear and in the basal regions of the OE. Differential expression of these proteins is suggested by dissimilar immunolabeling patterns. CRABPI appears to be present in olfactory receptor neuron (ORN) dendritic and axonal processes. To date, we see no evidence for CRABPII in ORNs. Presence of these binding proteins in the OE suggest that RA is functional in this tissue

195 Poster

VITAMIN A IN OLFACTORY MUCOSA AND ITS EFFECT ON GENE EXPRESSION IN NEURONS IN VIVO.

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A growing body of evidence supports the idea that retinoic acid (RA), an oxidized derivative of vitamin A (VA), is required for cells to commit to a neuronal phenotype. Since neurons are regenerated throughout adult life in the olfactory epithelium (OE), this tissue offers a unique opportunity to determine directly whether RA does influence neurogenesis in vivo. If RA is involved in neurogenesis in the OE, we would expect (1), to find RA or markers of RA metabolism in the OE and (2), that neurogenesis in the OE would be compromised in the absence of RA. Based on chromatographic analysis, we have determined there are measurable levels of retinoids in extracts of nasal mucosa. Based on analyses of tissues from mature, male rats nutritionally deprived of VA, we have determined that retinoid levels are negligible in nasal mucosa from VA-deficient (VAD) rats. Based on immunohistochemical analysis, we have determined that cytosolic and nuclear retinoid binding proteins are present in cells of the OE. Based on quantitative, reverse transcriptase- polymerase chain reaction (RT-PCR), we have determined that the mRNA expression levels for olfactory marker protein (OMP), a neuron-specific marker, are 40% of control levels in VAD rats. Together, our data suggest that (1), retinoid metabolism is active in postnatal rat nasal mucosa and (2), retinoids are affecting the maturation of olfactory sensory neurons in vivo.

196 Poster

ANALOGS THAT CROSS-ADAPT TO ANDROSTENONE MAY USE DIFFERENT OLFACTORY PATHWAYS

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A current hypothesis regarding olfaction suggests that odor coding involves the recognition of chemical ligands by olfactory receptors followed by activation of specific spatial patterns in the olfactory bulb. The olfactory system has a remarkable capacity to establish new afferent connections and restore sensory functions after denervation. This provides a unique opportunity to disrupt the mechanisms underlying coding and to examine the effects of disruption and restoration on odor perception. Recovery from denervation alters nerve projections to the olfactory bulb and odor quality perception after recovery from sensory denervation. However, the perceptual fate of structurally and/or perceptually similar odors following recovery after denervation and reinnervation is unknown. In this study, 5α-androst-16-en-3-one (androstenone) and its perceptual and/or structural analogs, 5α -androstan-3-one (androstanone) and 4-(4',4'-dimethylcyclohexyl)-2-methylcyclohexanone (DMCMC) provide olfactory stimulation. The advantage of using these latter compounds is their potential ability to cross-sensitize androstenone (AND), which will provide further insight into interactions in the olfactory system. It is hypothesized that analogs that cross-sensitized AND share common olfactory pathways with AND. Preliminary results demonstrate that exposure to either androstanone (ANA) and DMCMC can induced sensitivity to AND and both odorants are perceptually similar to AND in non-surgical mice. Different results were observed in mice who had recovered from a surgical ablation of olfactory nerve to both olfactory bulbs (BNX). Exposure to ANA 10 days immediately after surgery increased AND sensitivity, however exposure to DMCMC did not affect sensitivity to AND. In a generalization paradigm, all the BNX mice perceived ANA to be similar to AND. While some of the BNX animals perceived DMCMC to be similar to AND, others perceived DMCMC to be different from AND. These results suggest that ANA and DMCMC may be using different pathways of the olfactory system.

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197 Poster

TEMPORALLY REGULATED EXPRESSION OF LEUKEMIA INHIBITORY FACTOR RECEPTOR IN PRESUMED GLOBOSE BASAL CELLS AND ENSHEATHING CELLS FOLLOWING OLFACTORY BULBECTOMY IN MICE

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We are testing the hypothesis that infiltrating macrophages are a source of cytokines that regulate cell cycle progression leading to neurogenesis in the murine olfactory epithelium (OE) and regeneration of the olfactory nerve (ON) following olfactory bulbectomy (OBX)-induced neurotrauma. As previously reported, there was a transient increase in the BrdU labeling of globose basal cells (GBCs) that peaked at 3 days post-OBX and returned to near-control values by 20 days post-OBX. The mean number of infiltrating macrophages, which were identified by membrane expression of the F4/80 antigen, transiently increased, peaking at 3 days post-OBX and returning to near-control values by 20 days post-OBX. A cellular compartment analysis demonstrated that the greatest percentage of macrophages were localized in the OE at 16 hours post-OBX and in the ON at 3 days post-OBX. Because macrophages that infiltrate sites of neurotrauma in peripheral nerve may secrete leukemia inhibitory factor (LIF), we used confocal laser scanning microscopy to investigate the expression of its receptor. LIFR was transiently expressed by GBCs 2 days post-OBX, followed by its transient expression in ensheathing cells in the ONs 3 days post-OBX. Initial RT-PCR experiments demonstrated the presence of mRNAs encoding LIFR and gp130, which is a component of the interleukin (IL)-6/LIF receptor complex, together with LIF mRNA in isolates of total RNA from the nasal-olfactory epithelium of control and 3 day post-OBX mice. The results of our ongoing study indicate a role for LIFR and certain members of the IL-6 cytokine family, including LIF, in the regulation of GBC cell cycle progression leading to neurogenesis and of regeneration of the ON following neurotrauma.

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198 Poster

STEROID CONTROL OF CELL PROLIFERATION AND NEUROGENESIS IN THE OLFACTORY EPITHELIUM OF THE HAWK MOTH *MANDUCA SEXTA.*

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In insects such as the moth *Manduca sexta*, the adult olfactory epithelium develops *de novo* during metamorphosis. The presumptive antenna develops from imaginal disc tissue which grows predominately during the final larval stage. At the larval/pupal transition (pupation) the imaginal disc has developed as a long cylinder one cell layer thick, and is laid down upon the surface of the animal, secreting a cuticle shell in which the adult antenna develops. Adult antennal development is divided into several stages: proliferation, differentiation, morphogenesis, and maturation. About 72 hrs after pupation, the epithelium detaches from its cuticle (apolysis) to allow morphogenesis to occur unconstrained. Neurogenesis occurs during the proliferative and differentiative stages. In 1976 Sanes and Hildebrand published an exquisite developmental study of the *M. sexta* antenna and pheromone specific sensilla. In particular, mitotic events giving rise to olfactory neurons and support cells were identified as occurring during a relatively brief period of approximately 24-60 hrs after pupation, completing by the time of antennal apolysis. We have refined this observation to show that the mitotic events occur in a spatial and temporal wave, primarily during the third 24 hr period following pupation, and that this wave and the expression of certain pattern regulating transcription factors are regulated and sustained by ecdysteroids. These studies suggest that the timing of proliferation is linked to the steroidally regulated breaking of diapause (winter dormancy). These studies further suggest that, at least in *M. sexta*, the neuron/support cell clusters that underlay each sensillum may not be related from birth (sharing a common sensory mother cell), but rather establish associations following the proliferative period. These observations raise questions regarding the determination and regulation of cellular phenotypes, especially the coordinate expression of olfactory genes specific to given olfactory cell clusters and sensilla.

199 Poster

GROWTH AND PROLIFERATION IN THE ANTENNAL IMAGINAL DISC DURING THE FINAL LARVAL INSTAR OF THE HAWK MOTH *MANDUCA SEXTA.*

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In insects such as the moth *Manduca sexta*, the adult olfactory epithelium develops *de novo* during metamorphosis. The presumptive antenna develops from imaginal disc tissue that grows during the larval stage. At the larval/pupal transition (pupation) the imaginal disc has developed as a long cylinder one cell layer thick, and is laid down upon the surface of the animal, secreting a cuticle shell in which the adult antenna develops. The epithelium of the imaginal disc develops into the olfactory epithelium. Based on studies in *Drosophila*, imaginal discs are classically thought to initiate their development during embryogenesis. However, this may not be the case for all insects. To understand how spatial patterns are established in the developing adult olfactory epithelium, we are examining the development of the imaginal disc prior to the larval/pupal transition. We have characterized growth of the imaginal disc throughout the 8-9 days of the final larval stage, and are correlating this with mitotic activity. These studies are establishing a background against which to characterize the expression of patterning genes which, in turn, are expected to lead to the establishment of spatial domains within the olfactory epithelium.

200 Poster

ALTERATIONS IN OLFACTORY MUCOSAL DIFFERENTIATION AND PROLIFERATION INDUCED BY THE HERBICIDE ALACHLOR

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Chronic exposure of rats to the chloracetanilide herbicide alachlor is associated with the development of olfactory mucosal polypoid adenomas/adenocarcinomas. Prior to tumor development, marked changes occur in the proliferative and differentiation patterns of the olfactory mucosa (OM). The earliest lesions in the OM are small epithelial plaques in which the normal pattern of olfactory mucosal differentiation is disrupted. Altered differentiation does not appear to be the result of aberrent regeneration, as alachlor lacked direct cytotoxicity to the OM. In addition, the normal proliferative pattern of the OM is lost, with a significant number of S-phase nuclei (detected by BrdU immunohistochemistry) present throughout the OM, rather than only in basal cell layers. We are exploring several possible mechanisms of alachlor-induced olfactory epithelial alterations. First, one of the in vivo metabolites associated with alachlor exposure is a quinone imine, which would be capable of redox cycling; a possible sequela of this activity would be oxidative damage to DNA, resulting in mutations. Urine from control rats vs. those administered alachlor in the diet for 6 mo was analyzed for 8-hydroxy-2'deoxyguanosine (8OHdG), an indicator of excision repair of oxidatively damaged DNA. Alachlor-treated rats excreted >20X more 8OHdG than controls. The tissue localization of this response is currently under investigation, with preliminary evidence that the response occurs in the OM. To investigate the genetic basis of malignant transformation in the OM, we are comparing gene expression patterns by different stages of transformed epithelium. Preliminary data demonstrate greater levels of c-myb protein in alachlor-induced tumors that in control OM; this transcription factor is highly expressed during development, but at lower levels in the mature epithelium, suggesting that the OM may, in effect, be undergoing an alachlor-induced de-differentiation process. These results suggest that oxidative stress and c-myb dysregulation may be critical factors in alachlor-induced carcinogenesis.

201 Poster

EXPRESSION OF GALECTINS 1 AND 3 AND OLFACTORY MARKER PROTEIN (OMP) IN HUMAN OLFACTORY EPITHELIUM

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Due to their regulatory functions and modulating effects in various tissues, galectins, a family of endogeneous lectins, have been the subject of intensive research in the past years. Their appearance in a wide range of organisms has led to insights into mechanisms of cell regulation. Since the olfactory epithelium is a rare example of a regenerating nerval tissue, we examined the expression of galectin-1 and -3 in human olfactory epithelium. The expression pattern of galectin-1 and galectin-3 was investigated in relation to olfactory marker protein (OMP) using confocal laser immunofluorescence in human specimens and post-mortem biopsies. OMP-expression was found in olfactory receptor neurons (ORN) in the olfactory mucosa and in fibers of the olfactory nerve crossing the submucous connective tissue. Galectin-1 was expressed in both the connective tissue of the nasal cavity and in the basal layer of the olfactory epithelium. In contrast, galectin-3 expression was limited to cells of the upper third of the olfactory epithelium. Expression of both, galectin-1 and galectin-3, occurred in OMP-positive cells. However, between areas of galectin-1 and galectin-3 expression in the lower and upper portion of the epithelium, OMP-positive ORN did not stain for both galectins. Considering the potential role of galectin-1 and galectin-3 in cell differentiation and maturation, the differential localization of galectines in the olfactory epithelium appears to be consistent with a significant role of these molecules in the physiological turnover of ORNs.

202 Poster

NEUROGENESIS IN THE VOMERONASAL EPITHELIUM OF ADULT RATS: EVIDENCE FOR DIFFERENT MECHANISMS FOR GROWTH AND NEURONAL TURNOVER.

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The pattern of cell migration during neuronal turnover in the mammalian vomeronasal sensory epithelium (VN-SE) is controversial. In mice, pools of proliferating cells were detected at the edges and were described as migrating to the central region of the VN-SE (Barber and Raisman, 1978, Brain Res 141: 57-66). Recently, it has been reported in rats that dividing neurons are also present along the entire basal lamina of the VN-SE (Weiler et al., 1999, Eur J Neurosci 11: 700-711). Similarly, in marsupials, dividing cells have been observed not only in the margins but also in the center of the VN-SE, the latter of which have been demonstrated to migrate vertically and become neurons (Jia and Halpern, 1998, J Comp Neurol 400: 287-297). To investigate whether the process of neuronal turnover in placental mammals consists of horizontal and/or vertical migration and whether or not this process is common to mammals, adult rats were injected with bromodeoxyuridine (BrdU) and allowed to survive for different periods of time. The distribution of BrdU-labeled cells in the horizontal and vertical dimension of the VN-SE was analyzed as a function of time. Both horizontal and vertical migrations of BrdU-labeled cells were detected. Since cells in the central regions of the VN-SE migrate vertically and, as demonstrated by co-expression of Gi2α and Goα proteins and BrdU, become mature on day 1, it is very likely that these cells participate in neuronal turnover. Conversely, since cells in the margins of the VN-SE stop migrating horizontally on day 14, it is unlikely that these cells ever reach the center of the VN-SE. Since the VN-SE continues to grow during adulthood, it is likely that these cells constitute pools for growth.

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203 Poster

EVIDENCE FOR BIDIRECTIONAL CONTROL OF CELL PROLIFERATION IN THE OLFACTORY EPITHELIUM

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Although it has long been known that cells are continually produced in the olfactory epithelium, the regulation of this process remains unclear. Several lines of evidence indicate that cell proliferation rates can be down-regulated. For example, formation rates and neuron numbers are reduced when airflow through the nasal cavity is blocked. The present experiment was designed to determine if cell production rates could be *increased.* We used reversible external naris occlusion to decrease rates of proliferation, and then assessed the effects of the return of normal stimulation. Right external nares of experimental animals were occluded on postnatal day one with polyethylene plugs that were removed 20 days later. Tissue was collected at 3h, 24h, 48h, 5d and 10d following reopening. Animals were injected with bromodeoxyuridine (BrdU) 2h prior to sacrifice. Quantitative measures of the proliferating populations of globose basal, sensory, and sustentacular cells as well as epithelial thickness were made. Preliminary results suggest a sharp increase in the rate of neurogenesis between 24h and 48h post-reopening, tapering to normal levels by 5d. A surge of labeled globose basal cells preceded an increase in BrdU-immunoreactive olfactory sensory cells by approximately 24h. Labeled sustentacular profiles peaked early during recovery and quickly attained normal levels. The proliferation of these cell populations contributed to a full recovery of epithelial thickness by 5d. Taken together, these results show that stimulation of the olfactory epithelium can bidirectionally affect steady states of proliferation.

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204 Poster

THE POSSIBLE ROLE OF CASPASES IN OLFACTORY CELL DEATH

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We investigated the potential roles of three members of the interleukin-1-beta-converting enzyme (ICE) protease family (caspases) in apoptosis in olfactory epithelium. By RT-PCR analysis, the mRNAs of caspase 1 (ICE), caspase 2 (ICH-1) and caspase 3 (CPP32) were detected in olfactory mucosa obtained from normal adults, E19 fetuses and unilaterally bulbectomized rats. The transcript of caspase 2 disappeared in bulbectomized animals 3 and 5 days postoperatively, but reappeared 21 days postoperatively. This suggests that most of the caspase 2 transcript was in olfactory sensory neurons. We used TNF-α to induce cell death in organotypic cultures of E19 olfactory epithelium and assayed the ability of three caspase inhibitors to reverse the TNF-α effect. After 6 hrs of treatment with medium containing TNF-α , a 2.5 fold increase in apoptotic body number was observed throughout the olfactory epithelium. Pre-treatment of the cultures with either of two irreversible caspase inhibitors (Z-VAD-fmk, Ac-YVAD-cmk) for 4 hrs, followed by a 6 hr treatment with TNF-α plus an inhibitor, blocked TNF-α -induced cell death completely. Pre-treatment with a third caspase inhibitor (Z-DEVD-fmk) in the same treatment schedule reduced the numbers of apoptotic cells significantly but not to the same extent as Z-VAD-fmk or Ac-YVAD-cmk. Increasing the dose of any of the inhibitors reduced the numbers of apoptotic figures below those of control cultures, indicating the inhibitory response is dose dependent. Taken together, the results suggest that caspases 1, 2 and 3, and perhaps others that are blocked by the inhibitors we used, participate in TNF-α -induced cell death in vitro.

205 Poster

CASPASES 3 AND 9 CARRY A PRO-APOPTOTIC SIGNAL FROM SYNAPSE TO CELL BODY IN OLFACTORY RECEPTOR NEURONS

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Mature Olfactory Receptor Neurons undergo apoptosis when deprived of their target, the olfactory bulb. Caspases 3 and 9 have been suggested to mediate the terminal stages of neuronal apoptosis in vivo and in vitro. We can demonstrate that early pro-apoptotic signalling events in injured olfactory neurons result in an elevation of endogenous caspase 3 and 9 proenzyme expression. In the later stages of ORN apoptosis, caspase 9 is maximally activated (by cleavage) immediately prior to the maximal activation of caspase 3. We also demonstrate that the active caspase stimulus is initiated at the level of the lesion and carried in a caudo-rostral wave from the synapse back down the axon to the ORN cell body. As caspase 3 carries its pro-apoptotic signal from the olfactory bulb to the olfactory epithelium, it also cleaves the Amyloid Precursor Like-Protein, APLP2, as it travels through the ORN neuraxis. These data suggest that the major caspases responsible for neuronal apoptosis (3 and 9) are expressed in axons and can be activated axonally following deafferentation. In the context of a controlled partial bulbectomy, the pro-apoptotic caspase 3 and 9 signals can be initiated as proximally as the presynaptic membrane. During the retrograde activation of caspases 3 and 9, their downstream axonal targets also become cleaved. Caspases 3 and 9 are thus ideally positioned to mediate the balance between survival and apoptotic signalling pathways at every level of the developing and mature olfactory neuraxis.

206 Poster

THE IMPACT OF CHEMOSENSORY DYSFUNCTION ON QUALITY OF LIFE

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Clinical experience and informal surveys suggest that chemosensory dysfunction can impact substantially on quality of life (QOL). There has, however, been no systematic attempt to examine the relationship between QOL and various forms of chemosensory dysfunction. We have developed a questionnaire to assess the impact of chemosensory dysfunction on every day life using psychometric scales based on published models. The questionnaire also includes utility-based or time trade-off scales, again based on published models, to obtain a measure of the value placed by patients on smell and taste function. A preliminary version was mailed to a limited number of former patients of the Monell-Jefferson Taste & Smell Clinic (Breslin et al., 1997, *Chem. Senses*, 6:650). Based on patient responses and input from colleagues, we have revised and re-structured the questionnaire (to allow direct comparison of patient responses with those of healthy controls), and have now administered it to 105 patients as they have presented to our Clinic for evaluation. Patients also completed the short form of the Beck Depression Inventory to screen for generalized depression. Results are internally consistent, and indicate the importance of the chemical senses to QOL. For example, expressed concern about the ability to detect smoke, gas leaks and spoiled food was strongly related to the existence of measurable smell dysfunction, and extremely high among patients with such dysfunction. 20-33% of patients rated their mood and ability to enjoy food and social interactions as only fair to poor. These ratings were associated with general depression scores, which in turn tended to be higher among those with taste problems than those with smell problems. Finally, half of the patients were willing to spend 20% of their annual household income to correct their chemosensory dysfunction, and half had already spent at least $250 out-of-pocket (18% $1000).

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207 Poster

IMPACT OF OLFACTORY IMPAIRMENT ON QUALITY OF LIFE AND DISABILITY

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Over 2.7 million adults in the USA have chronic olfactory impairment. The extent to which such deficits affect patients' lives remains poorly defined. To determine whether olfactory loss impacts quality of life or level of disability, in 1999 we mailed surveys to 1292 patients evaluated at two university smell and taste clinics from 1984-1998. A total of 420 patients with documented olfactory impairment at the time of clinic visit completed the survey. In the survey patients were asked to rate their ability to smell on a scale of 0 to 10. Patients were assigned to one of two groups: "impaired smell" (n = 345) or "normal smell"(n = 75). There was no significant difference in age, sex, co-morbidity, education, work, or smoking status between the two groups. Responses to 15 questions regarding ability to perform common activities of daily living (ADLs) and 21 questions regarding quality of life issues were compared. The mean number of ADLs affected by olfactory loss reported by patients in the impaired group was 4.70, SD±3.56 and in the normal group 0.61, SD±1.58 ($p < .001$). The specific activities most commonly reported were: (impaired vs normal; p value) ability to detect spoiled food (75 % vs 12%; $p < .001$), gas leaks (61% vs. 8%; $p < .001$), or smoke (50% vs. 1%; $p < .001$), eating (53% vs. 12%; $p < .001$) and cooking (49% vs. 12%; $p < .001$). Among quality of life issues, the categories of safety and eating revealed a number of significant differences between the two groups. For hygiene issues, only the concern over body and breath odor differed between groups. Fewer subjects in the impaired group (50%) than in the normal group (87%) reported that they were satisfied with life ($p < .001$). The results of this study indicate a higher level of disability and lower quality of life for patients with impaired olfactory function.

208 Poster

THE USE OF LABELED MAGNITUDE SCALING FOR LONG-TERM CLINICAL ASSESSMENT

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The Labeled Magnitude Scale (LMS) is a recently developed alternative to classic absolute magnitude estimation. It is a semantic scale of perceptual intensity characterized by a quasi-logarithmic spacing of its verbal labels. In preparation for a long-term clinical study, we have evaluated the use of the LMS to track olfactory function over time in normal subjects. Seven subjects were tested 7 times over a 10-week period. In each of the seven sessions magnitude estimates for 5 concentrations each of Phenyl Ethyl Alcohol (PEA), lyral and NaCl were collected using the LMS. The five NaCl stimuli serve as cross-modal control stimuli.

Ratings for PEA and NaCl proved to be very stable over sessions. The ratings for lyral, however, showed more variability. Furthermore, the exponent of the psychophysical function for lyral was considerably smaller than that for PEA, and very close to 0. In the clinical study, we expect that as hyposmic subjects' smell function improves with treatment, there will be a corresponding increase in the estimated magnitude of the olfactory stimuli, but not of the gustatory ones. This makes lyral a less suitable candidate than PEA for tracking changes in olfactory function over the concentration range used.

209 Poster

RETEST RELIABILITY OF ALCOHOL SNIFF TEST

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Although an estimated sixteen million Americans suffer from olfactory deficits, olfactory ability is rarely assessed. A standardized test that is quick, cost effective and can be easily performed at the bedside, the Alcohol Sniff Test (AST), is a newly developed method of measuring olfaction. However, its short term, test-retest reliability has not been assessed in adults. To address this, we had thirty volunteers, all subjectively normosmic nonsmokers take the AST twice in the same setting with a three-minute interval between tests. The second test scores showed a significant reduction of olfactory ability compared to the first ($p = .0016$). Based on these findings, the use of the AST repetitively over a short time span to determine acute changes in olfactory ability is not recommended.

210 Poster

THE ALCOHOL SNIFF TEST COMPARED TO THE UNIVERSITY OF PENNSYLVANIA SMELL IDENTIFICATION TEST

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The Alcohol Sniff Test (AST), an easily performed clinical test of olfactory ability, has been validated in comparison to odor threshold tests but not in comparison to odor identification tests. In order to compare the AST to the most widely used odor identification test, the University of Pennsylvania Smell Identification Test (UPSIT), we had 21 patients with neurological or chemosensory complaints take both tests: their scores showed a correlation ($p = .01$, $r = .524$) between the two tests, suggesting that the simpler Alcohol Sniff Test should be evaluated further as to its validity as a substitute for the University of Pennsylvania Smell Identification Test in assessing olfaction in patients with neurological or chemosensory complaints.

211 Poster

DEVELOPMENT OF THE SMELL THRESHOLD TEST™ (STT): A COMMERCIALLY-AVAILABLE TEST OF ODOR DETECTION THRESHOLD SENSITIVITY

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Although the University of Pennsylvania Smell Identification Test (UPSIT; available commercially as The Smell Identification Test™) has proven useful in a wide variety of test situations, there is a need for a valid companion test of odor detection threshold sensitivity. In this paper, the development of such a test is described. This reliable and easy-to-administer test evolved from a detection threshold test that has been employed at the University of Pennsylvania Smell and Taste Center for over 15 years. Its features include (i) unique oval squeeze bottles that provide consistent stimulus delivery, (ii) non-liquid chemical stimuli, (iii) employment of odorants that do not elicit intranasal trigeminal nerve (CN V) responses, and (iv) a self-contained brief-case sized aluminum case and writing surface that can serve as a test table. The reliability of the Smell Threshold Test™, available from Sensonics, Inc., www.smelltest.com, equals or exceeds that of its predecessor. This test represents a further advance in quantitative testing of human olfactory function, and now makes well-validated standardized threshold testing available to the scientific and medical communities at large.

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212 Poster

THE DEVELOPMENT OF A COMPACT DIGITAL OLFACTOMETER

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MicroFab Technologies Inc. has been conducting clinical testing of a new technology for identifying dementing brain disorders, including Alzheimer's and Parkinson's and for differentiating them from other mental disorders. This method is based on detecting the olfactory deficits that are diagnostic of the dementing disease. This clinical trial has yielded very promising results.

MicroFab's Digital Olfactometer is based on current ink-jet technology. It utilizes a microdispenser which maintains an odorant volume dispensing resolution of 200 pL. Each dispensing channel is digitally and instantaneously (less than 10 ms cycle time) addressable. By presenting brief clouds of odoriferous vapor, the temporal integration (~100ms) of sensory responsiveness of the olfactory mucosa can be examined.

213 Poster

TRIMETHYLAMINURIA IN REFERRED PATIENTS WITH IDIOPATHIC BODY AND ORAL MALODOR

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The presence of a malodor in an individual with no apparent hygiene or diagnosable medical problems can be baffling for the health professional and frustrating for the patient. The odor-producing disorder trimethylaminuria(TMAU), produces this scenario for both clinicians and patients. Patients report foul body odors, halitosis, and/or dysguesia, which can produce social embarrassment and can only be temporarily relieved by normal hygiene procedures. TMAU is a genetically-mediated disorder, which appears to be inherited in an autosomal, recessive fashion. The presenting symptoms of TMAU stem from excess, unmetabolized TMA , a gas at body temperature and has a foul, fishy odor. At very low concentrations, it may only be perceived as foul or "garbage-like". Further, symptoms are often sporadic in occurrence and seemingly subjective. When coupled with a lack of knowledge of the disease and its etiology among health professionals, the result is often a diagnosis of poor hygiene, psychiatric problems and/or referrals to other specialists . We have diagnosed more than 50 TMAU-affected individuals in the past 10 years from more than 200 patients referred to our laboratory. During examination, patients may not present with any apparent "fish" or other noticable malodor: body malodor and/or fish-like odor have been encountered in only ~10% of these patients. All patients are administered the same diagnostic protocol, regardless of presenting symptoms, which utilizes analytical and bacteriological measures to determine the origin of a patient's complaints.

Patients with and without TMAU also present with halitosis caused by bacterial plaque on the tongue. We have begun to examine the genetics of the patients presenting to us with TMAU. Our results are consistent with those of other labs which report a variety of mutations and polymorphisms in the microsomal FMO3 liver enzymes which cause TMAU-symptoms.

214 Poster

ODORANT PLEASANTNESS, INTENSITY AND FAMILIARITY IN PATIENTS WITH SEASONAL AFFECTIVE DISORDER

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Because anhedonia is a cardinal symptom of major depression, because some evidence suggests a certain neuroanatomical overlap in olfactory and emotional processing, and because olfaction may modulate photoperiodic responses in certain species, we hypothesized that: 1) Compared to normal controls, depressed patients with SAD would report odorants to be less pleasant; 2) SAD patients would report odorants to be less pleasant in 'depressed' than in 'light treated' state; 3) In patients with SAD, depression scores would be negatively correlated with odorant hedonic ratings.

Twenty-four patients (17 females and 7males) with seasonal affective disorder, winter depression type, and matched controls, were studied during winter. Twenty-two patients completed the study. Their mood was rated using the SAM-SAD scale, and typical and atypical subscores were calculated. Fifteen odorants were presented successively, in a random order, birhinally, on a filter paper approximately one centimeter below the nostrils, for five seconds, with one minute for rating completion and two-minute intervals between stimuli. Subjects rated the odorants on visual analogue scales, assessing pleasantness from extremely unpleasant to extremely pleasant, familiarity from extremely unfamiliar to extremely familiar and intensity from undetectable to extremely strong.

We compared 'pleasantness', 'intensity' and 'familiarity' in patients vs. controls using Mann-Whitney U-tests and in patients, in 'depressed' vs. 'light-treated' conditions using Wilcoxon tests. We further analyzed, in patients, the degree of association between odorant ratings and depression ratings using Spearman's correlations.

No difference in 'pleasantness', 'intensity', or 'familiarity' was found between patients and controls and, in patients, between 'depressed' and "light-treated" conditions. No significant correlation was found between odorant ratings and depression ratings. Our results did not confirm a hypothesized olfactory anhedonia in depressed patients with SAD.

215 Poster

THE RELATIONSHIP BETWEEN THE LOSS OF PERCEPTUAL INTENSITY AND WATER SOLUBILITY IN SUBJECTS WITH COLDS

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This study examined the hypothesis that the magnitude of the decrease in olfactory ability that is usually seen during a cold (Chojnacki et al., Chemical Senses 19(5): 453, 1994) is related, in part, to an odorant's water solubility (Hornung et al., Chemical Senses 20(6): 710, 1995). Sixteen subjects with upper respiratory infections were confirmed to be hyposmic by scoring between 20 and 30 on the UPSIT. Subjects rated the perceptual intensity of 18 odorant stimuli with the Green Scale (Chemical Senses 18: 683-702, 1993). The test series was composed of 9 odorants chosen because of their water solubilities (Propionic Acid, Butyric Acid, Isopropanol - highly water soluble; Octanol, Hexanoic Acid, Pentanol - moderately water soluble; Hexanol, Trans-cinnamaldehyde, Heptanoic Acid - water insoluble). Each odorant was presented at two concentrations resulting in perceptual ntensities of about 40 and 20 as judged by normosmic control subjects without colds. Subjects with colds also used the Green Scale to rate the brightness of a series of lights. After the cold had resolved, subjects repeated the odorant and light intensity ratings. The perceptual intensity of the highly water soluble odorants was reduced by 36% in subjects with colds, whereas the intensity was reduced by 24% for the moderately water soluble odorants and only 12% for the water soluble odorants. The cold did not affect the light intensity ratings. We hypothesize that there is a direct relationship between an odorant's water solubility and the percent of incoming molecules that are sorbed by the narrowed nasal air passageways. As a result, for water-soluble odorants comparatively fewer odorant molecules reach the headspace above the olfactory receptors and so the perceptual intensity is more dramatically reduced during a cold.

216 Poster

TASTE FUNCTION IN XEROSTOMIA BEFORE AND AFTER SALIVA REPLACEMENT THERAPY

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Xerostomia (feeling of a dry mouth) may affect individual dietary habits, nutritional status, oral hygiene, speech, and decreased gustatory sensitivity. The present study specifically investigated effects of saliva replacement therapy on taste function. Whole-mouth gustatory function was assessed in 25 patients suffering from xerostomia (6 male, 19 female; age range 42-82 years) before and after 4-6 weeks of saliva replacement therapy using a preparation containing carboxy methyl cellulose. Results were compared to healthy controls matched for age and gender (6 male, 19 female; age range 42-82 years). Taste function was assessed quantitatively for sucrose, citric acid, sodium chloride, and caffeine. All patients easily detected the four taste qualities at the highest concentration. However, patients with xerostomia had significantly lower scores in the gustatory test compared to healthy controls .No correlation was found between duration of xerostomia or severity of the disorder. While therapy had no effect on taste function, saliva replacement lead to a significant improvement of other xerostomia-related symptoms. In conclusion, the study confirms previous work indicating that xerostomia is accompanied by decreased gustatory sensitivity. Results of this pilot study also seem to indicate that the routinely performed replacement of saliva has little or no effect on whole mouth gustatory function.

217 Poster

THE RELATIONSHIP BETWEEN OLFACTORY ACUITY AND CHRONIC SINUSITIS

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Nasal and sinus disease are one of the most common causes of olfactory loss, accounting from 15 - 27 % of patients presenting to taste and smell centers. Contrary to a sensory or neural loss a loss secondarily due to nasal and sinus disease is thought to be conductive, which means the odorant can not reach the olfactory epithelium and stimulate the appropriate receptors. Such patients often present because of impaired nasal obstruction, discharge, headache and recognize the loss of smell to be a predictable consequence. It has been shown that patients suffering from pathologies of the osteomeatal complex may suffer from olfactory disorders, but do not complain about nasal obstruction. Whereas no specific therapies have been found to be effective in the case of sensorineural loss, inflammatory or obstructive abnormalities in the nose impeding olfactory transport should certainly be amenable to further treatment.

Twenty consecutive patients suffering from chronic sinusitis and olfactory disturbances were evaluated before and after surgical treatment. Olfactory function testing was performed by means of a psychophysiological examination (using Sniffin' Sticks). All patients reported about a gradual onset of hyposmia (which was revealed in 18 patients, only 2 were completely anosmic). Patients were tested 3 to 6 weeks after surgery, complete recovery of the olfactory function (age and gender adjusted data) was observed in 75 % of our sample. 15 % improved in the performance of the olfactory function test although subjectively they did not recognize a change in daily life. Finaly two patients failed to show any improvement due to surgery. There was no prognostic parameter detected, to predict the effect of surgery on olfaction. Therefore we suggest olfactory function testing should be performed prior to nasal surgery in the same way as audiometry precedes any sort of ear surgery.

218 Poster

OLFACTORY FUNCTION AND CIRRHOSIS OF THE LIVER

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It has been noted that cirrhosis of the liver is occasionally accompanied by a reduced chemosensory ability. However, only little data on chronic liver disease and olfactory sensitivity are available (Burch R.E, 1978). In the present study we examined olfactory thresholds (T), odor discrimination (D) and odor identification (I) in patients with cirrhosis of the liver (CL, n = 50). Major aims were to investigate (a) whether cirrhosis of the liver (CHILD classification) is associated with changes in olfactory sensitivity, (b) whether there is a relationship between global psychometric measurements (Reitan A; Mini Mental State Examination, MMSE) and performance in the olfactory tests and (c) whether levels of zinc or bilirubine relate to olfactory function.

Independent of the CHILD classification the vast majority of CL patients healthy controls: 4.5 % of CL patients in our sample were anosmic, and 63.6 % were hyposmic. Only 31.8 % of CL patients were normosmic. All patients had olfactory scores equal to or lower than the 40th percentile (adjusted for age and gender). Reitan A test and MMSE exhibited a positive correlation with olfactory sensitivity; the highest coefficient of correlation was found for the odor identification test. Zinc levels were generally lower than normal but did not correlate with the degree of olfactory loss. Also, no such correlation was found for the serum bilirubin levels.

Finally, one of the most interesting finding was that CL etiology apparently had no influence on the degree of olfactory loss.

219 Poster

THE OLFACTORY SYSTEM IN LARYNGECTOMEES - CHEMOSENSORY EVOKED POTENTIALS AND PSYCHOPHYSICAL TESTING

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Introduction: Laryngectomees are often reported to be anosmic.It is still a question of debate whether this olfactory deficit is only due to a diminished transport of odorants to the olfactory epithelium or to an impairment of olfactory receptor cells. Material and Methods: We examined 25 laryngectomees psychophysically using the Sniffin'Sticks test battery. Moreover, all patients had to rate their subjective disturbance caused by the olfactory deficit on a visual rating scale. In 11/25 patients chemosensory evoked potentials were recorded additionally. Hydrogen sulphide was used as a specific olfactory stimulus and carbon dioxide was used as a specific trigeminal stimulus. Recording of evoked potentials was obtained by using an olfactometer. Results: Sniffin' Sticks testing classified 18 patients to be anosmic and seven to be hyposmic. However, sixteen patients subjectively complained very little about their smell deficit rating their disturbance below 3 (max. = 10) on the rating scale. Chemosomatosensory (carbon dioxide) evoked potentials could be recorded in all patients. Olfactory (hydrogen sulphide) evoked potentials could only be recorded in 7 out of 11 patients. In the 4 remaining patients no olfactory evoked potential could be seperated from background noise, although 2 out of 4 patients perceived in 4 stimuli out of 15 a liminal sensation of hydrogen sulphide. Conclusions: The psychophysical data revealed that the investigated laryngectomees suffered from functional anosmia or hyposmia. When using the olfactometer for stimulation, it could be demonstrated that many years after surgery (max. 22 years) the olfactory system was still functioning. When comparing subjective reports and olfactory evoked potentials it became obvious that olfactory evoked potentials can only be recorded in suprathreshold ranges, i.e. when there are not only just noticeable but rather clear olfactory sensations.The discrepancy between the olfactory deficits found in psychophysical testing and the lack of subjective complaints needs further elucidation.

220 Poster

OLFACTORY FUNCTION AND ADAPTATION FOLLOWING LONG-TERM OCCUPATIONAL EXPOSURE TO STYRENE

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Impairment of olfactory function in humans has frequently been associated with occupational exposure to volatile chemicals. Although animal toxicologic studies have found dose-related changes in the olfactory epithelium from exposure to many chemical agents, including styrene, few controlled studies relating current and historical exposures to comprehensive assessments of olfactory function have been undertaken. To investigate whether occupational exposure to styrene was associated with olfactory impairment, we examined olfactory function in a group of workers with a minimum of 4 years exposure to styrene in the reinforced-plastics industry (current mean exposure: 26 ppm, range: 10- 60 ppm; historic mean dose: 154.1 ppm-years, range: 13.8 - 328 ppm-years) and in a group of age- and gender-matched, unexposed controls. Both peripheral and central olfactory function were assessed using a standardized battery of clinical assessments that included tests of threshold sensitivity for phenylethyl alcohol (PEA), odor identification ability and retronasal odor perception. Odor detection thresholds for styrene were also obtained as a measure of specific adaptation to the ambient environment.

To evaluate any relationship between olfactory function and workplace exposure, each worker's olfactory assessment was examined with respect to their exposure profile, which was based on current and retrospective determinations of airborne styrene exposure.

No differences were observed between exposed workers and controls on any general tests of olfactory function. As expected, odor detection thresholds for styrene were significantly elevated among exposed-workers, consistent with exposure-induced olfactory adaptation.

Despite observations in animal studies that exposure to 20-50 ppm styrene (at or below currently acceptable workplace limits) produces lesions in the olfactory epithelium of rodents, the present study found no evidence among a cross-section of reinforced-plastics workers that current or historical exposure to styrene was associated with either self-reported or objective impairment of general olfactory function.

221 Poster

OLFACTORY QUALITY DISCRIMINATION DEFICITS IN SCHIZOPHRENIA USING THE "SNIFFIN' STICKS"

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Olfactory identification deficits in schizophrenic patients have been shown nearly across all published studies involving olfactory identification. Beside the verbal (lexical functioning as the response mode) and other complex cognitive aspects (recognition, and retrieval of a label or name) of such identification tests, olfactory identification involves olfactory functioning with respect to odor detection, quality discrimination and recognition memory. Except for olfactory acuity, there is a lack of research in these olfactory domains in schizophrenia.

Following the goal of a multivariate approach of olfactory functioning we used the "Sniffin' Sticks" for screening multiple types of olfactory measures in male schizophrenic patients. The "Sniffin' Sticks" comprises three tests of olfactory function, namely tests for odor threshold (n-butanol, testing by means of a single staircase), odor quality discrimination (triple forced choice discrimination task comprising 16 triplets; same/different judgement) and odor identification (16 common odorants, multiple forced choice from four verbal items per test odorant). Our ongoing study so far includes 13 male schizophrenic patients (DSM-IV) and 11 healthy male controls in the age between 19 and 35.

Preliminary main results show significantly reduced ability to qualitatively discriminate between odors in schizophrenic patients. Since odor detection and quality discrimination presumably require less cognitive processing than odor identification does, it can be argued that odor identification is more influenced by cognitive status than these other olfactory domains. A further possible explanation for the numerous findings of odor identification deficits in a variety of neuropsychiatric disorders, which therefore cannot be a specific "vulnerability marker" for schizophrenia alone, could be deficits in quality discrimination ability. Accurate performance on olfactory identification tasks is thought to require also intact quality discrimination ability.

Future research in olfactory functioning in schizophrenia and other neuropsychiatric disorders, including psychophysical measurements should be pursued along the lines followed other sensory systems, using a multivariate approach.

222 Poster

EFFECT OF THE NMDA ANTAGONIST CAROVERINE ON NON-CONDUCTIVE OLFACTORY DISORDERS: A PRELIMINARY STUDY

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Treatment of non-conductive olfactory disorders is an unsolved problem. The current clinical phase II trial focused on possible effects of the NMDA antagonist caroverine, which may act as a neuroprotective agent. Only patients with non-conductive olfactory disorders were included. A total of 51 patients received caroverine for one month (120 mg/d); 26 controls matched for age, gender, and duration of the olfactory loss were treated with zinc sulfate for the same period (400 mg/d). Evaluation of olfactory sensitivity was performed by means of a validated psychophyscial test kit ("Sniffin' Sticks") before and one month after treatment, i.e., testing included assessment of odor thresholds, and odor identification. When compared to baseline results, treatment with caroverine improved both odor thresholds ($F[1,33] = 9.248$, $p = 0.005$) and odor identification ($F[1,14] = 5.03$, $p = 0.042$) in anosmic patients; in hyposmics it also improved odor identification ability ($F[1,9] = 5.71$, $p = 0.041$). In contrast, zinc administration had no significant effect on olfactory function. Potential mechanisms for this effect may include reduced feedback inhibition in the olfactory bulb as a consequence of NMDA-antagonistic actions. Alternatively, antagonism of an excitotoxic action of glutamate may remedy ischemic lesions in the olfactory bulb. To establish the preliminary findings from this pilot study, a prospective multi-center, randomized, placebo-controlled trial in large groups of patients is currently under way.

223 Poster

FALLS FROM THE HOOD: A PREVENTABLE CAUSE OF CHEMOSENSORY DYSFUNCTION

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Head injuries are a common cause of chemosensory dysfunction especially amongst the young. Approximately five percent of patients suffering from head injuries have olfactory or gustatory loss which result in considerable morbidity. A frontal or occipital blow is usually the inciting injury. Loss of consciousness does not always occur. Anosmia typically results. Hypogeusia, and rarely ageusia, can also occur. A review of the literature provides several possible explanations for these findings. We present three cases of chemosensory loss resulting from head injury incurred by falling from the hood of a moving vehicle. This paper illustrates the potential of chemosensory dysfunction and its associated morbidity to occur from this easily avoidable etiology. We reiterate the importance of falls from the hood as a cause of smell and taste loss and emphasize education as a preventative measure of this type of injury.

224 Poster

EFFECTS OF MEDIOTEMPORAL AND INSULAR LESIONS ON TASTE AND SMELL

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Rationale: Our PET studies of healthy subjects have localized human primary and secondary olfactory and gustatory cortices. We report here patient JL, who had presented with intractable epileptic seizures and whose MRI scans revealed an extraordinary pattern of damage that included bilateral anteromedial temporal lobe and posterior orbitofrontal atrophy, and severe left insular atrophy. As this damage invaded primary gustatory (PGA) and olfactory cortices, JL's chemosensory function was explored with psychophysical and PET methodologies, in addition to her basic neuropsychological tests. JL underwent surgery for epilepsy, and some tests were repeated after operation.

Methods: Memory was assessed with four tasks, and odor detection thresholds were tested. Taste was tested with detection and recognition thresholds, whole-mouth intensity and pleasantness judgments, and unilateral intensity judgments on the tongue. One smell and two taste conditions were used in the PET study. After surgery the taste tests and some memory tests were repeated.

Results: Global memory deficits and anosmia were present before surgery, with some further memory loss postoperatively. Gustatory detection thresholds were normal before and after surgery. Recognition thresholds were moderately elevated preoperatively, while afterwards a taste agnosia was observed. The laterality tests showed lower intensity estimates with left-sided stimulation, ipsilateral to the insular lesion. Taste intensity and pleasantness judgments were abnormal. These last measures were not retested.

PET study (pre-op only): Olfactory stimulation elicited no activation in the region of olfactory cortices. Gustatory stimulation elicted unilateral activity in the (intact) right PGA. Activation was observed in the left secondary gustatory cortex during tasting of a pleasant taste, indicating functional tissue bordering the lesion.

Discussion: Before surgery, despite the absence of left PGA, only mild taste deficits were observed, more pronounced on the left, consistent with a postulated ipsilateral taste pathway and right hemisphere predominance for taste. The significance of postoperative losses will be discussed.

225 Poster

ANOSMIA DUE TO INHALATIONAL ZINC: A CASE REPORT

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A 47 year old married white male with no past history of chemosensory difficulties experienced the sensation 'as if a cold were coming on' and tiredness. No fever, rhinorrhea, nasal congestion, cough or malaise were present. To prevent the development of an upper respiratory tract infection, Zicam nasal inhaler was applied as per manufacturer's specifications, one application per nostril with delivery of approximately 250 micrograms of zinc per inhalation.

He immediately experienced severe right periorbital pain and anosmia. The pain resolved in one day, but the anosmia persisted. Despite treatment with Zithromax and Prednisone, the olfactory ability did not return, precipitating a visit to a chemosensory clinic one month later. At that time, olfactory testing demonstrated anosmia with an UPSIT score of 20, Alcohol Sniff Test of 6 cm, and olfactory threshold to Carbinol at an irritant level greater than 35 decismels in both nostrils. Isovaleric acid, 2,3-Butanedione, Pentadecalactone, Phenylethyl Alcohol, and Tetrahydrothiophene were absent at 25 decismels. Isobutyl Isobutyrate and L-Carvone were intact at 25 decismels.

The patient never developed a cold, nor had any underlying illnesses which could account for the chemosensory deficits. Ionic Zinc instilled directly on the olfactory epithelium appears to be the pathogen in this patient's smell loss. Given the above, further investigation of the olfactory effects of Zicam nasal inhaler is warranted.

226 Slide

NEW GENE SPECIFICALLY EXPRESSED IN RHESUS MONKEY TASTE BUDS BY DIFFERENTIAL SCREENING OF TASTE BUDS AND ADJACENT EPITHELIUM CDNA LIBRARIES FROM LASER CAPTURE MICRODISSECTED TISSUE

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Getting pure populations of taste buds suitable for molecular analysis has hampered the characterization of genes specifically expressed in taste cells. As an approach to solving this problem we prepared specific cDNA libraries from small amounts of taste cells and surrounding epithelium isolated by laser capture microdissection (LCM) and report here the discovery of a rhesus monkey novel gene (rmSTG) specific to taste cells found by differential screening of the cDNA libraries.RNA in situ hybridization shows the preferential expression of this gene in taste buds from circumvallate, foliate and fungiform papillae of the tongue.Genome walking and rapid amplification of cDNA ends of the cloned fragment revealed a gene with two exons and one intron. This prediction was further supported by northern analysis of circumvallate papillae showing a transcript of 1.3 Kb as established in the gene model.The predicted gene encodes a protein of 314 amino acids bearing a N-terminal signal peptide and cleaveage site suggesting protein secretion and an extracellular role for rmSTG in taste cell physiology , although function as a membrane bound protein is not excluded.RT-PCR and northern analysis with different non-taste organs showed no expression, pointing to a very specialized function of the protein.Blast search analysis shows that the human homolog is localized in the recently completely sequenced HLA class I region in chromosome 6p21 in the main susceptibility locus of psoriasis vulgaris and was found by another group to be expressed in epidermal keratinocytes but not in other organs (tongue not included) by RT-PCR.

227 Slide

A NOVEL METABOTROPIC GLUTAMATE RECEPTOR FUNCTIONS AS A TASTE RECEPTOR

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Sensory transduction for many taste stimuli such as sugars, some bitter compounds, and amino acids is thought to be mediated via G-protein coupled receptors (GPCRs). Although GPCRs have previously been cloned from taste tissue, until now none has been expressed functionally. Nor have ligands that activate these receptors been found. The identification of candidate GPCRs as taste receptors hinges on the ability to demonstrate that such receptors respond to known taste stimuli at appropriate concentrations. Monosodium L-glutamate (L-MSG), a natural component of many foods, is an important gustatory stimulus believed to signal dietary protein. We have recently described a novel GPCR cloned from rat taste buds that may be a taste receptor for L-MSG. We have functionally expressed the receptor in CHO cells. The receptor couples negatively to a cAMP cascade and displays an unusual concentration-response relationship for the taste stimulus, L-glutamate. Importantly, the receptor is also activated by L-AP4, a compound that mimics the taste of MSG. We have termed the novel receptor taste-mGluR4. The similarities of its properties to MSG taste suggests that taste-mGluR4 is a taste receptor for glutamate.

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228 Slide

CLONING AND FUNCTIONAL CHARACTERIZATION OF GENES EXPRESSED IN GUSTDUCIN-POSITIVE TASTE RECEPTOR CELLS

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Gustducin is a transducin-like G protein selectively expressed in about 20% of taste receptor cells. Multiple lines of evidence from in vitro and in vivo experiments have shown that the α-subunit of gustducin (α-gustducin) is critical to the transduction of responses to bitter and sweet compounds. To clone and identify other components of the α-gustducin-mediated taste signal transduction pathways, single cell cDNA libraries were constructed from α-gustducin-positive and -negative taste receptor cells. ~45,000 plaques from α-gustducin-expressing taste cell cDNA libraries were differentially screened with probes from α-gustducin-positive vs. -negative taste cells. We isolated 600 clones which were preferentially expressed in α-gustducin-positive taste receptor cells. DNA sequence analyses of the 600 clones enabled us to categorize them as housekeeping genes, cell markers, development- and differentiation-related genes, transcription factors, signal transduction components, and novel sequences. Among the known clones were two G protein β subunits, Gβ 1 and Gβ 3. Among the novel clones was a previously unknown G protein g subunit (Gγ 13). Gene expression profiling and immunohistochemistry revealed that Gβ 3, Gγ 13 and phospholipase Cβ 2 (PLC β 2) coexpressed absolutely with a -gustducin in taste receptor cells. Using biochemical studies we showed that Gγ 13 interacts with a -gustducin, and that gustducin heterotrimers consisting of α-gustducin/Gγ 13/Gβ 1 were activated by taste cell membranes plus bitter denatonium. Using quench flow assays and anti-Gγ 13 antibodies we demonstrated that Gγ 13 mediates the denatonium-induced increase of inositol trisphosphate (IP3) in taste tissue. Based on the present work and previous studies, we conclude that gustducin heterotrimers transduce responses to bitter and sweet compounds via α-gustducin regulation of phosphodiesterase and Gβ γ 13 regulation of PLCβ 2.

229 Slide

MAMMALIAN BITTER TASTE RECEPTORS

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Taste is a major mode of sensory input in mammals, and bitter taste plays an important role in rejecting harmful substances. We have identified a new family of G-protein-coupled receptors that are expressed in subsets of taste receptor cells. These receptors (T2Rs) are organized in the genome in clusters, and map to several loci that influence bitter taste perception both in mice and in humans. The T2Rs are exclusively expressed in taste receptor cells that contain gustducin, a G protein which has been implicated in bitter taste signaling. Notably, taste receptor cells express multiple T2Rs, suggesting that these cells are capable of recognizing a structurally-diverse range of tastants. We developed a heterologous expression system to show that T2Rs function as bitter taste receptors. A human and a mouse receptor (hT2R4 and mT2R8) responded to the bitter tastants denatonium and 6-n-propyl-2-thiouracil, and a mouse receptor (mT2R5) responded to cycloheximide. Mouse strains deficient in their ability to detect cycloheximide have amino acid substitutions in the mT2R5 gene, and these changes render the receptor significantly less responsive to cycloheximide. We also expressed mT2R5 in insect cells and demonstrated cycloheximide-dependent activation of gustducin. Together, these results validate T2Rs as bitter taste receptors. Since a single taste receptor cell expresses multiple T2Rs, these findings provide a compelling explanation for the uniform bitter taste that is evoked by many structurally-unrelated toxins.

230 Slide

RANDOM DISTRIBUTION OF GUSTATORY SENSITIVITIES ACROSS RAT TASTE RECEPTOR CELLS AND BRAINSTEM NEURONS

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Several transduction mechanisms have been demonstrated in mammalian taste cells, but little is known about their distribution within and across receptor cells. We recorded whole-cell responses of rat fungiform taste cells maintained within an intact tongue epithelium in a modified Ussing (MU) chamber, which allowed us to flow tastants across the apical membrane while monitoring the activity of the cell with a patch pipette. Stimuli were: 0.1 M sucrose, 0.032 M NaCl, 0.1 M KCl, 0.1 M NH_4Cl, 3.2 mM HCl, and 3.2 mM quinine hydrochloride (QHCl). The cells were adapted to distilled H_2O flowing over their apical surfaces. In voltage-clamp configuration, cells showed voltage-activated outward currents, characteristic of taste cells. Application of tastants to the apical membrane resulted in reversible inward or outward currents; those over 5 pA were considered reliable responses. Sucrose and QHCl always elicited outward currents, associated with conductance decreases. NaCl, KCl, NH_4Cl, and HCl always produced inward currents, accompanied by increases in conductance. Each of 45 cells was tested with all four of the basic stimuli; 24 of these were also tested with KCl and NH_4Cl. Of the 45 cells, 13 (28.9%) responded to only one, 15 (33.3%) to two, 11 (24.4%) to three and 4 (8.9%) to all four of the basic stimuli; 2 cells (4.4%) responded to none of the four (but did respond to KCl and/or NH_4Cl). A stochastic analysis of the distributions of responses showed that combinations of sensitivities across these cells did not differ from that expected from an independent and random distribution, as shown previously for rat chorda tympani fibers (M. Frank and C. Pfaffmann, *Science* 164:1183-1185, 1969). Analysis of the activity of cells in the nucleus of the solitary tract showed a similar random distribution, but with greater breadth of responsiveness.

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231 Slide

A CATIONIC CHANNEL IN THE BULLFROG TASTE RECEPTOR CELLS DIRECTLY GATED BY BITTER-TASTE SUBSTANCES

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We reported previously that quinine activates a cationic current in bullfrog taste receptor cells under the whole-cell recording condition. In the present study we made experiments to elucidate the mechanism generating this current. We found that the channel activity was actually induced by bitter substances in an outside-out patch membrane in which none of the second messenger candidates or its precursors (cyclic nucleotides, ATP or GTP) were added. This observation led us to examine an idea that any second messenger systems are not essential for this bitter response. Indeed, we confirmed that the G protein cascade does not seem to be involved in channel gating, because 1) the response was recorded >10 min after the patch excision (all soluble factors residing on the cytoplasmic side of the membrane must have been washed away), 2) GDPβ S (1 mM) added to the cytoplasmic side did not suppress the quinine-induced channel opening, and 3) GTPγ S (1 mM) did not induce a spontaneous channel opening. All these observations show that there is an ionic channel that is directly gated by bitter substances. The quinine-induced current was dose-dependent in the concentration range of 0.1 to 1 mM ($K_{1/2}$, 0.52 mM). The channel was cation selective with the permeability ratio (P_{Na}:P_K:P_{Cs}) of 1:0.48:0.39. The unitary conductance was 9.2 pS in a nominally Ca^{2+}-free solution, and 4.5 pS in a 1.8 mM Ca^{2+}-containing solution. The concentration range of quinine, the cation permeability ratio, and the unitary conductance and its Ca^{2+}-dependence were almost identical to those of the quinine-induced whole-cell current reported previously. These identical properties indicate that the channel current observed in the excised membrane is the constituent of the whole-cell current. We therefore conclude that the bitter-induced cationic current flows through the ionic channel that is directly gated by bitter substances.

232 Slide

DETECTION OF DIETARY FAT BY THE GUSTATORY SYSTEM: BEHAVIORAL AND ELECTROPHYSIOLOGICAL PROPERTIES OF LINOLEIC ACID IN RATS.

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Behavioral studies have shown that adding fat, in the form of corn oil, to a rat's diet increases intake. One hypothesis speculates that in the oral cavity, salivary lingual lipase may reduce fat into free fatty acids (FFAs). Gilbertson (Am J Physiol, 1997) demonstrated that linoleic acid (LA), the principal FFA in corn oil, can modulate ion channels in isolated rat taste receptor cells. To further assess the ability of the gustatory system to detect fat, we conducted condition taste aversion (CTA) and whole nerve electrophysiological studies. Behaviorally, rats learned to discriminate 28μ M LA following a LiCl-induced CTA test. Bilateral transection of the chorda tympani (CT) nerve eliminated the ability to discriminate LA in a CTA paradigm. However, bilateral CT transection did not impair the general ability to learn a CTA as demonstrated in subsequent CTA tests using corn oil and sucrose. These behavioral results imply the CT has a necessary role in transmission of LA gustatory information to the CNS. Next, integrated CT electrophysiological responses were examined during lingual application of LA. LA was presented both as a concentration series (90-4.5μ M) and with NaCl and Glucose + Saccharin (G + S) solutions. Examining the integrated neural signal showed the CT was unresponsive to a broad range of LA concentrations. A 30s pre-treatment rinse of 90μ M LA followed by either NaCl (500mM) or G + S (30g + 1.25g/1l water) failed to show any modulation of responses to the tastants. Comparison of responses to NaCl (250mM) and G+S (15g + 0.63g/1l water) with and without 90μ M LA revealed no modulation of tastant responses. Lack of whole nerve responsiveness to LA does not exclude the CT as a means of transmitting LA gustatory information. More sensitive measures of individual CT neural subsets may reveal neural responsiveness to LA that is masked in the integrated nerve response.

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233 Slide

CHEMOSENSORY ALTERATION OF BRAIN ACTIVITY DURING MATH TASKS

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Previous research has demonstrated that odors administered before or during linguistic tasks often produce disruptions in performance or alter the pattern of brain activity associated with the task. Magnetoencephalographic studies have indicated that some odor processing is associated with activity in the superior temporal gyrus (STG) and this activity is more consistent on the left side of the brain. That area of the brain has often been associated with language and other symbolic processes and is thought to be specialized for the temporal parsing and encoding of information. If odors require the parsing and temporal encoding features of the STG, this overlap may be the reason that odors interfere with language tasks.

To test this hypothesis, ten undergraduate students were tested in a math task while odors were presented. We hypothesized that odors presented briefly during the math task should interfere with the solution in only the exact solution phase since symbolic processing is being used. Furthermore, the presentation of an odor mixture should produce the greatest effect since it requires more neural resources for parsing.

Three odors (vanillin 13%, PEA 20%, and equal parts mixture of the two) were administered via a constant flow olfactometer. Odor administration lasted 0.2 sec and was synchronized with inspiration. The math problem followed the odor offset and was presented for 0.3 sec. All of the math problems were simple addition problems such as 17 + 23. Brain activity was recorded throughout the experiment. Amplitude data were submitted to analysis of variance. The results indicated a significant interaction of task (exact vs. estimate) with odor and pattern of brain activity. Further analysis of this effect indicated that only the mixture produced a difference during the exact solution task. These findings lend strong support to the idea that odor parsing interferes with symbolic cognitive processing

234 Slide

OLFACTORY EVENT-RELATED POTENTIALS IN DEMENTIA

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This study addresses the aim of developing the olfactory event-related potential (OERP) as an objective measure of olfactory function that can be clinically useful in signaling dementing illness. The initial event in Alzheimer's disease (AD) is the appearance of plaques and tangles in entorhinal and transentorhinal cortex, which suggests olfactory functional impairment in AD. The study goals were: first, to examine OERPs in AD patients compared to age-matched normal controls, and second, to compare OERPs to auditory ERPs in AD patients relative to controls. Participants were 12 persons diagnosed with Probable AD using the NINCDS-ADRDA criteria and the DSM-III-R criteria, applied by senior staff neurologists from the Alzheimer's Disease Research Center at UCSD and 12 age/gender-matched controls. Average DRS for the AD patients was 119, indicating mild to moderate dementia. OERPs and auditory ERPs were elicited with a single stimulus paradigm. Latency was significantly longer for AD patients than for controls at P3 ($F = 34.1$, $p.001$), in response to olfactory stimulation. The effect size (eta2) for the latency difference between the AD patients and the controls for the P3 component at Cz in response to olfactory stimulation was .63; whereas in response to auditory stimulation it was .27. The latency differences between the Alzheimer's patients and the age-matched controls were strikingly larger (200 ms) than the latency differences between the two groups for the auditory P3 (50 ms), suggesting the potential clinical utility of the olfactory ERP in the assessment of dementia.

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235 Slide

HOW GOOD IS YOUR SENSE OF SMELL? AWARENESS OF OLFACTORY ABILITY IN PATIENT GROUPS.

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The sense of smell acts as an important detection system in warning people of the presence of spoiled food, smoke, natural gas, or other chemicals. Impairment in the sense of smell reduces the personal safety of individuals with olfactory loss. Naturally, the potential for danger to go undetected is substantially greater when a person is unaware of an olfactory loss. Nordin, Monsch, Murphy (1995. J. Gerontology, 50B(4), 187-192) report lack of awareness of threshold changes in elderly individuals (both normal and Alzheimer's patients) in contrast to accurate perception of sensitivity in younger sinusitis patients. But does awareness of olfactory ability change with age, etiology of loss, or are other factors at work? Further, would patients be better at estimating their loss when ability was measured with identification, given that olfactory identification performance is generally less variable than threshold performance? These questions led us to examine the data from 203 patients seen at the Smell and Taste Disorders Clinic for accuracy of estimation of olfactory identification performance on the Olfactory Confusion Matrix (OCM). Although patients self-rated estimates of ability with a gross category scale (Normal, Impaired but not absent, No ability, Highly sensitive), 42% of our sample was unable to do so accurately. There was no difference between specific etiologies of loss or age groups in estimating olfactory loss. However, depression and anxiety were measured in 85 of these patients, and depression scores were significantly higher in those patients with inaccurate perception (one-tailed t-test, $p = 0.05$). These findings are consistent with general trends in depression in which individuals tend to view the self negatively, and underscore the need for careful olfactory testing due to inaccuracies in self-perception.

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236 Slide

EFFECTS OF BREASTFEEDING CHEMOSIGNALS ON THE HUMAN MENSTRUAL CYCLEG

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Although the ovarian cycle has been the focus of most pheromonal studies to date, females of many higher primates spend a large portion of their reproductive life spans in birth cycles of conception, pregnancy, and lactation, rather than solely in spontaneous unfertilized ovarian cycles (Altmann et al., 1987). To date, there is little research on the effect of compounds from nursing women and their infants on the fertility of other women. In rodents, birth cycle pheromones from lactating females and their pups modulate the estrous cycle and behavior of female conspecifics (McClintock, 1984; Mennella and Moltz,1989).

Breastfeeding chemosignals combined breast and axillae pads from 26 lactating women. These pads contained mothers' milk, maternal body odors, and, most likely, compounds from infants, as mothers nursed during collection periods. In a randomized, double-blind, within- and between-subjects study design, we determined how exposure to such breastfeeding chemosignals modulated the ovarian cycles of 47 recipient women. Throughout the baseline cycle, all women applied carrier pads under their noses at least four times daily. During the experimental cycle, approximately half of the subjects received the breastfeeding chemosignals and the remainder received the carrier. There were no significant differences in cycle lengths between these two groups of women during the baseline cycle (χ^2 = 1.5, *P* 0.47). However, during the experimental cycle, those women who received the breastfeeding chemosignals had atypical cycle lengths when compared to the cycles of the carrier control group (χ^2 = 8.1, *P* 0.02). That is, those whose baseline cycles were less than 28 days became shorter and those whose baseline cycles were greater than 29 days became longer during the experimental cycles. The cycles of women who received the carrier did not significantly differ from baseline. We explored predictors of direction of change and how cycle phase characteristics influenced these significant differences in cycle length.

238 Slide

INFLUENCE OF KNOWLEDGE ON THE PERCEPTION OF EVERYDAY ODORS

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In a cross-cultural study on the perception of everyday odors we found a close correlation between familiarity, intensity, and pleasantness scores, indicating a possible influence of experience or knowledge on odor perception (Distel et al., Chem. Senses 24:191,1999). To investigate this more directly, we presented everyday odorants (in sniff bottles) under two conditions: (A) without information about the odor source, and (B) together with the veridical name of the odorant. In both conditions we asked subjects to rate the intensity, pleasantness, and familiarity of the odors on a scale from 0 to 10. In condition A we also asked subjects to rate the certainty of knowing the odor (from 1-5) and to identify the possible source, and in condition B, to rate how well the odor fitted the name provided (from 1-5). Two groups of 38 subjects (medical students) participated. Each group rated half the 24 odorants in condition A and the other half in condition B so that each odorant was rated equally in A and B. Providing the veridical name enhanced scores for intensity, pleasantness and familiarity by 0.7, 1.25, and 1.0 points, respectively, and this effect was significant (p 0.025, Wilcoxon test). If subjects were able to identify the odor source (28% of presentations in condition A) the enhancing effect was similar for the intensity ratings (p 0.04), and even more pronounced for the pleasantness judgements (p 0.0001). In contrast, when odors were judged not to fit the name provided (61% of presentations in condition B), they were judged to be less intense and less pleasant than when name and odor were perceived to coincide (5 vs. 6.75 and 6 vs. 7.5 points, respectively; both p 0.0001). These results indicate that knowledge of the odor source and perceptual expectations may strongly influence odor perception.

237 Slide

DECREASED OLFACTORY ABILITY IDENTIFIED IN SUSCEPTIBLE FARM WORKERS

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Anecdotal reports from patients and other clinicians suggest that farm workers may have decreased olfactory ability.

Attendants of an agricultural trade show in central Nebraska were invited to complete a questionnaire assessing farm work experience, health status and olfactory ability. Subjects also completed a 12-item odor identification test. Statistical analysis was performed on the number of correctly identified odorants for each subject (adjusted for age and sex) against each environmental exposure item on the questionnaire. The group includes 405 subjects, mean age 50±15 years, 191 females and 214 males.

319 subjects report active participation in farm work, 82 do not. Average scores for the olfactory test in these two groups are 9.3 and 10.1 respectively (= 0.2). 117 subjects report a flu-like illness after working on the farm, 259 do not. Those with such symptoms score a mean number correct of 9.1, those without, 9.8 (p = 0.07). Although subjects who report handling crops score about the same as those who do not, subjects with nasal symptoms after handling various grains score significantly lower. Scores for subjects with chronic nasal or sinus problems (n = 80) or nasal allergies (n = 125) are not significantly different from healthy subjects.

In this study, the olfactory ability of farm workers is not greatly different from non-farm workers. However, a significant decrease in olfactory ability is found in those with a history of flu-like illness after working on the farm. Decreased olfactory ability in subjects with a history of nasal symptoms after handling various grains (but not in asymptomatic grain handlers) suggests that the inflammatory effects of grain dust can decrease olfactory ability in susceptible individuals. Since there is no significant olfactory loss in subjects reporting chronic nasal or sinus problems or allergies, we conclude that this grain-dust-exposure olfactory loss is more likely neural than conductive.

239 Poster

MOLECULAR CHARACTERIZATION OF ODORANT RESPONSIVE CULTURED HUMAN OLFACTORY CELLS

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The ability of olfactory cells to proliferate in vitro has allowed researchers to use cultured cells as model systems for the investigation of olfactory function. To study human olfaction, we established cultured cell lines from adult human olfactory tissue obtained using an olfactory biopsy procedure and demonstrated their ability to respond to odor stimulation using calcium imaging techniques. Under specific growth conditions, these cultured human olfactory cells respond to odorant mixes that have been previously shown to elicit intracellular calcium ($[Ca^{2+}]_i$) changes in mature human ORNs (Rawson, N.E. et al. J Neurophysiol. 77, 1606-1613, 1997). As in the human ORNs, these $[Ca^{2+}]_i$ changes were reversibly blocked by inhibitors of the olfactory signal transduction cascades. To assess the developmental time course of structural and functional characteristics, we assayed odorant sensitivity using imaging techniques and protein expression at several times after plating. In order to determine which molecules are expressed by odorant-responsive cells, we also isolated single cells which had previously responded to odorant stimulation with changes in $[Ca^{2+}]_i$, or which expressed olfactory marker protein (OMP) immunoreactivity, and assayed mRNA expression using single-cell molecular techniques. We tested for the expression of OMP and olfactory receptors. We also tested isolated non-olfactory neurons from the same cell line to characterize the pattern of mRNA expression specific to each cell type.. By combining functional assays with molecular characterization it will be possible to evaluate the relevance of specific molecular markers to functional maturation of cultured human olfactory neurons.

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240 Poster

A NOVEL ISOLATION SYSTEM FOR HUMAN OLFACTORY RECEPTOR CELLS

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The study of human olfactory receptor cells (ORCs) has proven more difficult than in their lower vertebrate counterparts, primarily due to the limited accessibility of isolated cells. At best, current biopsy techniques on live humans yield a small number of cells and restricted topographic information. Consequently, a novel recovery system for ORCs from postmortem humans has been pursued. Morphologically identifiable cells can be obtained in this manner. Olfactory receptor cells exhibit well-defined cell bodies, dendrites, olfactory knobs with visualized cilia, and occasional axonal extensions. Ciliated respiratory cells have well defined columnar shapes with actively beating cilia. Perforated patch recording techniques on receptor cells reveal voltage-dependent inward and outward currents. These cells can also respond to specific olfactory stimuli with membrane current changes. While results are preliminary, these isolated ORCs appear to be similar to those obtained from biopsies on live humans. Apparently the olfactory epithelium is a privileged site postmortem, kept moist by the overlying mucous and oxygenated by their epithelial surface location. The potential benefits of this isolation system are numerous, including a greatly increased number of receptor cells isolated, topographical mapping capability, and no morbidity to the host.

241 Poster

OLFACTORY NEURON SUBTYPES DISPLAY UNIQUE AMINO ACID PROFILES BUT OVERLAPPING ODOR SENSITIVITIES

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The olfactory organs of the squid *Lolliguncula brevis* are bilateral auricular structures located posterior and slightly ventral to each eye, in a position to sample odorants as the animal breathes. Five subtypes of olfactory sensory neurons (OSNs) have been identified morphologically in the pseudostratified olfactory epithelium. We classified OSN subtypes according to their immunocytochemically determined amino acid components and found 4 unique amino acid profiles. Across several animals, amino acid profiles consistently identified the same morphological subtypes of OSNs. The type 4 OSN is the only subtype whose olfactory sensitivity has previously been studied in detail. Application of the appropriate odorants to type 4 cells elicits excitatory (glutamate) or inhibitory (betaine, dopamine) responses. To obtain information about the other cell types, we applied agmatine (AGB), an ion channel permeant probe that has been used to label odor-activated ORNs in lobster, crab and zebrafish. Application of odors and AGB to the squid olfactory organ allowed visualization of odor-stimulated cells across the entire epithelium. Approximately 4-5% cells in the olfactory epithelium were labeled when exposed to AGB alone. The most stimulatory odor, 100 μ M l-alanine, resulted in labeling of 10-14% of the ORNs. Other odors increasing labeling above levels stimulated by AGB alone included glutamate, arginine and proline. When odor labeling was superimposed on amino acid profiles, we found that odor responsivity was not limited to a specific cell type. Collectively, these data indicate that morphological or biochemical differences in squid OSNs do not limit the ability of cells to respond to specific odorants.

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242 Poster

VISUALIZING ODOR RESPONSES OF MOUSE OLFACTORY RECEPTOR NEURONS IN AN INTACT EPITHELIAL PREPARATION

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In contrast with rapid progress in the molecular biology of olfaction, there is little physiological data to characterize the odor response properties of different populations of olfactory receptor neurons (ORNs) as well as their distributions in the epithelium. In order to study these properties as they relate to the coding mechanisms underlying odor discrimination and recognition at the epithelial level, we have developed an intact epithelial preparation from the mouse, in which odor responses of hundreds of ORNs can be monitored simultaneously by calcium imaging techniques. When a swatch of epithelium was loaded with calcium-sensitive dye, calcium green-1, the dendritic knobs of ORNs appeared as bright spots in an *en face* view. They responded to odor stimulation by showing increased fluorescence intensities, which were measured for individual knobs. Our results indicated that subsets of mouse ORNs respond to different odors with distinct patterns, and a single ORN can respond to odors with distinctly different chemical structures. Sometimes the ORNs responding to the same odor were clustered, although in other cases they seemed to be distributed randomly. We observed neurons tuned specifically to different functional groups such as alcohol, aldehyde and fatty acid, when tested by applying chemical compounds with similar carbon chain length. For a given odor, more ORNs were recruited when the concentration of the odor was increased. The mouse ORNs were able to distinguish between two pairs of enantiomers (± carvone and ± limonene) in a concentration-dependent manner. Our method offers an efficient way to map responses of a group of neurons in the epithelium in a spatially defined manner under approximately in vivo physiological conditions.

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243 Poster

IDENTIFICATION OF MULTIPLE ORS THAT RECOGNIZE SPECIFIC ODORANTS

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In mammals, odorant detection is mediated by ~1000 different odorant receptors (ORs), which are encoded by a multigene family. Each OR gene is expressed by ~1/1000 olfactory sensory neurons, suggesting that each neuron expresses only one OR gene. The discrimination of odorants presumably derives from the different ligand specificities of the ORs. However, OR specificities have been difficult to ascertain, due to problems in expressing ORs in heterologous cells. We devised an alternative approach in which we first employed calcium imaging to identify mouse neurons responsive to various odorants, and then identified the OR genes expressed by individual responsive neurons using a two-step PCR procedure. For test odorants, we used a series of n-aliphatic odorants with different carbon chain lengths and, for each chain length, different functional groups. In several different types of experiments, we confirmed that individual neurons express one OR gene each, and that the OR sequences we amplified from neurons were not derived from genomic DNA. Our results showed that a single OR can recognize multiple odorants that share identifiable structural features, and that an individual odorant is recognized by multiple ORs with diverse protein sequences. However, different odorants were detected by different combinations of ORs, providing direct evidence for previous proposals that ORs are used in a combinatorial fashion to encode odor identities. Nearly identical odorants that are perceived as having dramatically different odors in humans were recognized by different, but often overlapping, sets of ORs, raising interesting questions about the roles of individual ORs in conveying perceived odor qualities. We are now extending these studies to other odorants with varied structures. For one odorant, only 9 neurons in >4000 tested were responsive, 3 of which expressed the same OR gene.

244 Poster

TOPOGRAPHY OF PROJECTIONS OF OLFACTORY NEURONS EXPRESSING HIGHLY RELATED ODORANT RECEPTORS

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Detection and discrimination of odorous molecules is based on specific odorant receptor proteins located in the ciliary membranes of olfactory sensory neurons. The repertoire of genes encoding such receptors is extremely large, numbering as many as 1000 genes for some mammals. To get insight into how the system is designed to encode information about a stimulus, the axonal projection pattern of olfactory neurons expressing distinct genes from a subfamily of highly related receptor genes (mOR37) was analyzed. A gene targeting strategy in mice allowed the coordinated translation of the receptor along with a marker protein, permitting to visualize the cells including their axonal projections. Using either taulacZ or tauGFP as axonal markers two different receptors could be visualized in the same individual by double labeling. Each gene was expressed in a distinct subset of olfactory neurons in the nasal sensory epithelium. Analyzing their axonal projections revealed that all cells expressing the same receptor project their axons onto a common glomerulus. The different populations target distinct glomeruli which are all grouped within a restricted domain of the olfactory bulb. Analysis of a large number of bulbs revealed that the relative positions of these glomeruli are not fixed but display local permutations.

245 Poster

H-LACZ6, A MOUSE MODEL TO STUDY THE EXPRESSION OF ODORANT RECEPTOR GENES ?

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In this study we characterize the mRNA expression pattern of OR-Z6, a new odorant receptor gene that we recently cloned. The OR-Z6 gene was initially discovered in the genomic context of the mouse line H-lacZ6, a transgenic line that was generated to study the function of the olfactory marker protein (OMP) promoter. The expression pattern of the reporter gene lacZ in the olfactory epithelium of H-lacZ6 mice exhibited features that were similar to the mRNA expression pattern seen for some odorant receptor genes. We subsequently cloned the odorant receptor gene OR-Z6 from the genomic region that flanks the transgene insertion site. Using X-gal staining and in situ hybridization in tissue of H-lacZ6 mice we have shown that olfactory neurons that express the OMP-lacZ transgene and/or the OR-Z6 receptor gene show a similar distribution and zonal restriction in the olfactory epithelium (Pyrski et al. AChemS 1998, 1999).

We now analyze the expression of both genes in the main olfactory bulbs. In this study we address the question whether axonal projections of olfactory neurons that express beta-galactosidase and/or OR-Z6 mRNA terminate onto identical or neighboring glomeruli. We present new data that might reveal insight into the mechanisms that direct the expression of odorant receptor genes.

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246 Poster

MOLECULAR MODEL OF A MOUSE OLFACTORY RECEPTOR REPLICATES EXPERIMENTAL ODOR RESPONSES FOR ALIPHATIC ALCOHOLS AND ACIDS

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An olfactory receptor (OR) can respond to multiple odor molecules, but the mechanisms for this selectivity remain unknown. There is presently no experimental crystal structure available for ORs. We have built an atomic-level structural model for mouse OR S25 (Malnic et al., 1999) by combining the density map for the related G protein-coupled receptor bovine rhodopsin with first principles computational methods recently developed at CalTech. We validated these methods on bacteriorhodopsin, a 7-transmembrane domain protein of known structure, and found that they predict the crystal structure within experimental resolution. We then used the methods to predict the binding pocket and interaction energies for 24 compounds tested by Malnic et al. (1999). The predicted odor-binding site resembles the epinephrine binding site of the beta-arenergic receptor and involves residues previously predicted to bind odors. The two compounds predicted to bind optimally in the model corresponded to the two observed experimentally to elicit response. The S25 model is the first to correctly predict differential receptor responses to a broad panel of potential agonist compounds. The results suggest mutation and ligand binding studies on S25 to elucidate the molecular interactions between olfactory receptors and ligands. They should also provide the framework for a long-awaited *in silico* approach to receptor characterization.

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247 Poster

CHARACTERIZATION OF MOUSE OLFACTORY RECEPTORS THAT RECOGNIZE A COMMON ODORANT MOLECULE

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Recent studies in characterizing orphan olfactory receptors revealed that odorant receptors, themselves, possessed unique receptive ranges based on structural determinants in odorant molecules such as differences in chain length, terminal groups, and positions of functional groups. In order to examine the specificity and diversity of odorant receptors that recognize a particular odorant of interest, we have developed a functional cloning strategy by combining calcium imaging and single cell RT-PCR techniques. The functional identification of receptors from single cells that responded to a common odorant molecule revealed that the receptors, which recognized the same odorant, were widely diversified according to phylogenic analyses based on the primary amino acid sequences. The receptors that recognize a spicy smell, eugenol, are less diverse than the ones which recognize more simple molecules like cresol, possibly because the recognition of a more complex molecule requires a binding site that is relatively more conserved among the receptors. One receptor for carvone is closely related to the receptor for limonene, a molecule that is structurally similar to carvone, while one of the other carvone receptors shows proximity to one of the receptors for cresol, a molecule that has minimum structural similarity with carvone. These studies suggest that multiple receptors for a certain odorant recognize different epitopes on the target odorant molecule, and that the phylogenic analyses of odorant receptors do not provide clear information for predicting candidate ligands or identifying residues involved in ligand binding. Nonetheless, our present studies and those of other researchers provide a clue for deciphering complex combinatory mechanisms of odorant-receptor interactions and tuning mechanisms that allow various odorants to be discriminated by the odorant receptors in the olfactory system.

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248 Poster

PROTEIN STRUCTURE PREDICTION FROM SEQUENCE INFORMATION: APPLICATIONS TO THE CHEMICAL SENSES

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The atomic structure of a protein provides critical information on its function. This is particularly true in the chemical senses, where the information coding and perceptual qualities of olfactory and taste molecules frequently depend on their shapes and the structures of the receptors they bind. Despite the importance of protein structure, direct information is often unavailable due to the difficulties of protein crystallization. We have developed a complementary computational approach to predict protein structure from multisequence information. The method, based on correlated mutation analysis (Goebel et al, 1994, Proteins 18:309-317), scans multisequence families for pairs of amino acids that mutate in tandem. It then assesses the structural fit between such amino acids, based on likelihood scores derived from hundreds of known structures in the Protein Data Bank. The method predicts pairs of amino acids that are distant in the sequence but likely to form close contacts in the tertiary structure. The results provide distance constraints that can be used to predict how the protein folds. Predictions for the sweet-tasting protein thaumatin and its homologs were 45% accurate, or 23.6 times better than random (BTR) prediction. Analysis of neural cell adhesion molecule (domain 1) was 22% accurate (6.5 BTR). Tests on 118 protein families yielded a mean accuracy of 15% (6.7 BTR) with a stringent 4.5A cutoff, 20% (4.1 BTR) with a moderate 6A cutoff, and 42% (2.9 BTR) with a relaxed 10A cutoff. The results show promise for computer-based protein structure prediction. The next step is to adapt the methods for membrane proteins important in olfactory and taste transduction, such as olfactory receptors.

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249 Poster

STATISTICAL EVALUATION OF OLFACTORY RECEPTOR NEURON RESPONSE TO CHEMICAL STIMULATION

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We introduced a novel non-parametric statistical method for action potential rate analysis in olfactory receptor neurons (ORNs). Prior to chemical stimulation the spontaneous firing rate of ORNs is fairly constant, leading, under the assumption of independence of events, to a uniform distribution of action potentials. After the stimulation, the ORNs firing rate is higher for excited and lower for suppressed neurons. The analysis is based on the properties of the cumulative distribution function (cdf) of action potentials. The slope of the cdf is directly related to the action potential firing rate. Prior to the stimulation, action potentials are uniformly distributed and have linear cumulative distribution with a specific slope. After the stimulation, the slope of cdf is higher for excited and lower for suppressed neurons compared to their prestimulus slope. The local firing rate of action potentials is estimated as a slope of the regression line in selected neighborhood of 5 to 11 consecutive action potentials. From the distribution of the estimated slopes in prestimulus data, a test interval of expected slopes is constructed using 5th and 95th percentiles. After the stimulation the cfd slopes are compared with the limits of the test interval. Slopes outside the test interval are treated as significantly higher (excitation) or lower (suppression) than expected. The beginning of the receptor cell response is determined as the time of the first significant slope, the response duration is the time difference between the last and first of the consecutive significant slopes. As a measure of the response intensity, the largest (excitation) or smallest (suppression) slope during the significant response is divided by the median prestimulus slope. Summary plots that presentated a large number of olfactory receptor neuron responses enabled identification of cell clusters that have similar response characteristics.

250 Poster

CLONING OF AN *AEDES AEGYPTI* ODORANT-BINDING PROTEIN FROM AN ANTENNAL EXPRESSED SEQUENCE TAG (EST) LIBRARY

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Since their discovery in moths, Odorant Binding Proteins (OBPs) have mostly been described in lepidopteran and few in other Insect orders like Hymenoptera, Hemiptera or Diptera. In the latter case, *Drosophila melanogaster* was the source of several molecules involved in odorant processing such as OBP-related proteins and now odorant receptors. Aedes aegypti is another well spread dipteran in which the host attraction behavior is well studied but not described at the molecular level. This is the reason why we generated a cDNA library from 200 *Aedes aegypti* male antennae. The strategy used for this cloning was to over represent small transcripts (between 800 and 1200 base pairs) in order to preferentially clone abundant mRNAs such as the OBPs. This Expressed Sequence Tag (EST) project produced 154 ESTs. Among them, 44 cDNAs were found to be unique sequences and one was shown to belong to the OBP-related protein (OBPRP) family. This abundant protein shares common features with several other proteins such as the olfactory specific proteins OS-F and ABPX of *Drosophila melanogaster* and *Bombyx mori*, respectively. The other proteins encountered in our screening were either already described or ones not corresponding to proteins found in any databases.

251 Poster

SELECTIVE BLOCKADE OF CHEMOSENSITIVE TRIGEMINAL AFFERENTS BY GUANETHIDINE

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Guanethidine is a chemical agent commonly used in the study of adrenergic neurotransmission and for the induction of chemical sympathectomy. During recent experiments in which guanethidine was used to eliminate sympathetic transmission in rats, we observed an incidental loss of trigeminal response to nicotine, suggesting a nicotinic acetylcholine receptor (nAChR) blocking effect for this compound. Guanethidine has already been suggested to have a blocking effect on nAChRs in the rat gastric fundus (Blommaart et al., 1999 Br J Pharmacol. 128:903-908). To further investigate this possible blocking effect, we are studying the effects of guanethidine administration on the trigeminal nerve response to nicotine and cyclohexanone. The peripheral receptors of the trigeminal system in the nasal cavity are free nerve endings arising from the nasopalatine and ethmoid branches of the trigeminal nerve. These chemosensitive AD and C fibers respond to a variety of chemicals and are scattered throughout the respiratory epithelium. Our previous research indicated that chemosensitivity of these fibers to nicotine was mediated by neuronal nAChRs functioning as peripheral chemoreceptors (Alimohammadi and Silver, Chem Senses, in press).

Multiunit neural recordings were obtained from the ethmoid nerves of Sprague-Dawley rats in response to nicotine and cyclohexanone. Vapor phase nicotine (12.5 ppm) and cyclohexanone (450 ppm) were delivered to the rats' nares via an air-dilution olfactometer. The magnitude of the trigeminal nerve response to nicotine decreased after the administration of guanethidine, whereas response to cyclohexanone remained unchanged. The present results are similar to previous results obtained using neuronal nicotinic acetylcholine receptor blockers specific for the $\alpha 4\beta$ 2 and α 3β 4 neuronal nAChR subtypes, suggesting a possible neuronal nAChR blocking effect for guanethidine in the rat nasal cavity.

252 Poster

REPELLENT COMPOUNDS STIMULATE INCREASES IN INTRACELLULAR CALCIUM IN CULTURED CHICK TRIGEMINAL NEURONS.

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Behavioral studies indicate that there are significant differences between taxa in the responses to chemical stimuli applied to the trigeminal (TG) field. For most mammalian species tested, capsaicin (CAP) is a particularly effective aversive stimulus. Birds, on the other hand are behaviorally insensitive to CAP and instead respond very strongly to methyl anthranilate (MA). Mammals respond to MA but only at high concentrations. In order to better understand the underlying mechanisms and the comparative differences between mammalian and avian responses to noxious TG stimuli, we determined the responses of cultured chick TG neurons to a prototypical pain-inducing compounds. In addition to CAP and MA, we chose histamine (HIST), acetylcholine (ACh), bradykinin (BK), and serotonin (5HT). Tested over the range of concentrations of MA from 3μ M to 3 mM, neurons showed an incremental increase in intracellular calcium. The response threshold was approximately 10 μ M, a concentration consonant with behavioral studies. Although CAP is a behaviorally inactive compound in avian species, chick TG neurons responded to CAP at relatively low concentrations (10 μ M). Very few neurons showed an increase in intracellular calcium to HIST (10μ M), BK (1 μ M), ACh (10 μ M) and 5HT (10 μ M), concentrations that are normally stimulatory to rat TG neurons. Supported in part with funding from the USDA, National Wildlife Research Center.

253 Poster

CAPSAICIN SELECTIVELY MODULATES VOLTAGE-GATED SODIUM CURRENTS (VGSC) IN RAT TRIGEMINAL GANGLION (TG) NEURONS THROUGH CAMP AND PKC MEDIATED PATHWAYS.

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Capsaicin, the pungent ingredient in hot pepper, initially causes a burning sensation by activating Na+ and Ca2+ permeable ion channels that evoke action potentials (APs) in a subset of TG nociceptors. Subsequently APs are difficult to evoke which is why capsaicin has analgesic properties. Whole-cell patch clamp, EIA, and single cell PCR measurements were done on TG neurons to uncover the mechanisms underlying capsaicin-induced desensitization. Of the four types of APs that characterize TG neurons (I-IV), the APs be inhibited by capsaicin only in Types I and II that are characterized by their activation by capsaicin, and their long duration. Measurements of VGSCs (I-V, h( ), and use dependence) revealed that capsaicin primarily effect inhibited Na+ conductance. This inhibition occured only in neuron Types 1 and II that contain the TTX-R subunits (SNS and NAN) and TTX-S subunits (A1 and RPN4). Moreover, the vanilloid receptor antagonist, capsazepine (CPZ) prevents capsaicin-induced desensitization, suggesting desensitization follows receptor activation. EIA studies in TG neurons revealed that capsaicin increased cAMP levels in an CPZ-inhibitable manner. In patch-clamped capsaicin-sensitive TG neurons both CTP-cAMP and PDBU, a PKC agonist, decreased Na+ currents. We conclude that there are two pathways (cAMP and PKC) by which capsaicin can selectively but indirectly modulate the activity of capsaicin-sensitive neurons. This work was supported by NIH DC-01065 and the Philip Morris Corporation.

254 Poster

ELECTRICAL RESPONSES TO VANILLIN AND CARBON DIOXIDE IN NASAL MUCOSA OF RATS INJECTED WITH 3-METHYLINDOLE

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Intranasal stimulation with volatile chemicals elicits negative mucosal potentials generated by either olfactory receptor cells or trigeminal nociceptors, and most volatiles can activate both receptor systems. Data from human studies suggest that vanillin mainly, if not exclusively, induces olfactory sensations, whereas carbon dioxide primarily causes trigeminal activation. We studied potentials evoked by vanillin (35% v/v) and carbon dioxide (65% v/v) in rats intraperitoneally injected with 300 mg/kg of the olfactotoxin 3-methylindole (3-MI). In this dose, 3-MI has been shown to produce destruction of nasal olfactory tissue and olfactory deficits estimated in behavioral experiments. Chemical stimuli of 1 s duration were delivered into the nasal cavity via an olfactometer in a constantly flowing (2 l/min) air stream with controlled temperature and humidity. Responses to vanillin and carbon dioxide (20 presentations each; interstimulus interval 90 s) were recorded (electrode resistance about 20 kOhms) from the nasal septum at 4, 8, and 16 days following treatment with 3-MI. Potentials obtained in each rat were averaged and the mean amplitude for different post-3-MI intervals was determined. Basal responses were recorded in control rats injected with corn oil in place of 3-MI. After 3-MI administration, the response to vanillin was markedly depressed during 8 days and significantly increased in amplitude by day 16. There was a positive correlation between the response amplitude and post-3-MI time. In contrast, the response to carbon dioxide, although decreased, did not differ significantly from the basal response and showed no significant changes during the time of observation. These results suggest that the response to carbon dioxide, unlike that to vanillin, is less affected by 3-MI. The question arises as to whether this difference reflects a relative tolerance of the intranasal trigeminal system to 3-MI.

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255 Poster

GNRH IS PRESENT IN RAT NASAL GLANDS FOLLOWING GONADECTOMY

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Gonadotropin releasing-hormone (GnRH) modulates olfactory neuron responses to odors. We hypothesize that GnRH from the nervus terminalis reaches the surface of the chemosensory mucosa via nasal gland secretions. We have previously shown that Bowman's glands of tiger salamanders contain GnRH immunoreactivity. To further test our hypothesis that GnRH accesses the olfactory receptor neuron dendrites via mucous secretions and that this phenomenon can be generalized to other species, we conducted a second immunocytochemical study in rats. Rat nasal glands showed little GnRH immunoreactivity. However, if animals were gonadectomized, which causes GnRH neurons to release large amounts of the peptide, nasal glands on the nasal septum adjacent to and within the vomeronasal organ demonstrated intense GnRH immunocytochemical labeling. In rodents this area receives robust nervus terminalis projections. This study demonstrates that: 1) GnRH is present in the nasal glands of mammals, 2) the steroidal state of the animal affects the amount of GnRH available in nasal glands, and 3) there are species differences in regard to the amount of GnRH in nasal glands under normal conditions.

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256 Poster

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE ADULT HUMAN VOMERONASAL ORGAN

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The vomeronasal organ (VNO) in humans has long been regarded as absent or functionally irrelevant. For example, the nerval connection between the VNO and the accessory olfactory bulb has been reported to degenerate during the second half of pregnancy. Further, the data on the organ's occurrence in adult humans exhibit considerable variation. The aim of this study was to immunohistochemically evaluate the neurogenic potency of the epithelium (PGP 9.5, NSE, OMP), its proliferative capacity (PCNA, Ki-67), and the relation to extracellular matrix components (e.g., hyaluronate receptor CD44). Negative results for neuronal markers including OMP indicate that (1) there is little, if any, neuron-like cell activity, and (2), there may be no functionally significant nerval connection between the VNO and central brain structures. On the other hand, (3) proliferation antigens in nuclei of basally located cells of the VNO are regularly expressed. Positive reactions for CD44 demonstrate a role in differentiation and migration of VNO cells. In summary, the vomeronasal epithelium can be characterized as a highly differentiated organ. It is fully developed and exhibits a unique "pseudostratified" epithelial structure. Hence, its actual function in adult humans awaits further clarification.

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257 Poster

THE OLFACTORY EPITHELIUM OF JUVENILE CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) BECOMES SENSITIZED TO A SPECIFIC F-PROSTAGLANDIN WHEN FISH ARE TREATED WITH AN ANDROGENIC STEROID HORMONE.

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Although F-prostaglandins (PGFs) have been shown to function as sexually dimorphic pheromones in many fish species, the role of this class of compounds in channel catfish has not yet been examined despite the importance of this species as a model for chemosensory function. The objectives of this study were to determine whether the catfish olfactory epithelium is sensitive to specific PGFs and if so, whether this sensitivity is influenced by treating the fish with an androgenic steroid hormone. We recorded electro-olfactogram (EOG) responses from androgen-treated and untreated juvenile catfish (n = 5 for each) that were sequentially exposed to five stimuli: 1) 10^{-8}M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$); 2) 10^{-8}M 15-keto $PGF_{2\alpha}$ (15K, a metabolite of $PGF_{2\alpha}$); 3) 10^{-8}M 13,14-dihydro-15-keto $PGF_{2\alpha}$ (13,14DH, a metabolite of 15K); 4) 10^{-5}M L-arginine (an amino acid standard); and 5) appropriate controls. For androgen treatment, fish were implanted with pellets containing methyl-testosterone (MT), a synthetic androgenic steroid, at least two weeks prior to experimentation. Neither MT-treated nor untreated fish detected $PGF_{2\alpha}$; however, both groups responded equally well to 13,14DH (about 30% of the standard, $P < 0.05$). In contrast, fish treated with MT responded to 15K (22% of standard, $P < 0.05$), while untreated fish did not. This specific pattern of responsiveness to these three PGFs has not been previously reported in a fish. Presently, we are conducting dose-response and cross-adaptation experiments to characterize the sensitivity and specificity of responsiveness to 15K and 13,14DH. In conclusion, this study demonstrates that channel catfish detect water-borne PGFs and confirms other studies that show androgens to induce specific pheromonal sensitivity in cyprinid fishes. Future studies are necessary to determine if sensitivity to PGFs in channel catfish is associated with a pheromonal function and if the physiological basis of the MT-induced sensitivity to 15K reflects upregulation of olfactory receptors.

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258 Poster

VOLTAGE-DEPENDENT AND ODOR-ACTIVATED CURRENTS IN OLFACTORY RECEPTOR NEURONS OF SEA LAMPREYS

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Olfaction is important for feeding, migration and reproduction in sea lampreys (*Petromyzon marinus*). Migrating adults appear to use unique bile acids, excreted by larval sea lampreys, as a cue for selection of a spawning stream (Teeter, 1980, Can. J. Fish. Aquat. Sci. 37:2123; Bjerselius et al., 2000, Can. J. Fish. Aquat. Sci.,in press). In addition, sexually mature males, and probably females, release pheromones that attract conspecifics of the opposite sex (Teeter, 1980) and presumably function in pair-formation and release of spawning behavior. L-Arginine (L-arg) and the larval bile acids, petromyzonol sulfate (PS) and allocholic acid (ACA), elicit marked EOG responses in migrating sea lampreys. We used the perforated-patch recording technique to identify voltage- and odorant-activated currents in olfactory receptor neurons (ORNs) from sea lampreys captured during the 1999 spawning migrations in the Cheboygan River, MI and St. Mary's River, Ontario. Many ONRs displayed spontaneous action potentials, as well as discharges to injection of depolarizing current and application of odorants. A variety of voltage-dependent currents were observed, including: a transient, TTX-sensitive Na^+ current; a high-voltage-activated, nimodipine-sensitive Ca^{2+} current; a delayed rectifier K^+ current; an inactivating K^+ current; a Ca^{2+}-activated K^+ current; and an inward rectifier K^+ current. Of 51 neurons stimulated with L-arg, 4 displayed action potentials under current-clamp, 17 responded with activation of a cation conductance that reversed near 0 mV, and 4 displayed odorant block of the inwardly rectifying conductance. PS was applied to 20 ORNs, eliciting activation of a cation conductance in 3 cells, block of the inward rectifier current in 1 cell and action potentials in 1 cell. Application of the phosphodiesterase inhibitor, IBMX, resulted in depolarization in 2 of 6 neurons. These results provide the basis for experiments to examine the mechanisms of pheromone transduction in a primitive vertebrate.

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259 Poster

THE OTHER SENSE IN OLFACTORY SEARCH BEHAVIOUR: RESPONSES OF MECHANOSENSORY LATERAL LINE AFFERENTS TO FLOW.

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The New Zealand long-fin eel, *Anguilla dieffenbachii*, may use chemical and hydrodynamical cues to follow an odour plume. This multimodal search strategy may be more efficient than one based on chemical signals only. Hydrodynamical stimuli are detected by the mechanosensory lateral line system. The lateral line is composed of hair cell receptors, which either form sub-epidermal canal or superficial neuromasts. Neuromasts are innervated by primary afferents originating from the octavolateralis complex (8th cranial nerve). In this study we recorded extracellularly from anterior lateral line afferents while stimulating the eel with unidirectional water flows between 0.5 to 4 cm/s. These flow rates approximate stimuli encountered by eels in their natural environment. Primary afferents showed a wide range of response magnitudes to a low background flow. Eighty percent of all afferents were flow-sensitive and increased in response magnitude with increased flow rate. Afferents showed little or no adaptation to prolonged stimulation, i.e., monitored changes in flow rate precisely. These results suggest an important contribution the mechanosenory lateral line system could provide in fish olfactory search behaviour.

This study was supported by a Marsden Fund to JCM, and a scholarship to AGC by the New Zealand National Institute of Water and Atmospheric Research.

260 Poster

ELECTRON IMMUNOCYTOCHEMICAL STUDY OF GI2α AND GOα IN THE VOMERONASAL SENSORY EPITHELIUM

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Recent studies have demonstrated that there is a layered organization to G-protein and G-protein coupled receptor expression in the sensory epithelium of the vomeronasal organ. Gi2α and Goα expressing neurons are localized to the apical and basal halves of the receptor cell layer. However there is little information about the localization of these G proteins in the fine structure of the vomeronasal organ. To examine this question, electron microscopy was used to observe the pattern of immunoreactivity for antibodies to the G protein subtypes on cell surface structures in the vomeronasal epithelium. Vomeronasal receptor cells are bipolar neurons whose apical dendrites reach the epithelial surface and form a knob-like structure covered with microvilli. At first, we studied the distribution of antibodies to Gi2α and Goα in control animals. Strong immunoreactivity was localized to the microvilli and the knob-like surface structures of receptor cells. This localization is similar to that reported for putative pheromone receptors. No immunoreactivity was found on the microvilli or surface membranes of supporting cells. The colocalization of G proteins and pheromone receptors on vomeronasal receptor cells suggests the possibility of a functional coupling between the two molecules in the pheromone transmission cascade. Electron microscopy was also employed to examine changes of Gi2α immunoreactivity during regeneration and recovery from nerve transection. At recovery day 30, immunoreactivity was localized to the surface of the receptor cells even though there were no microvilli. At recovery day 60, there were a few microvilli on the cell surface of receptor cells and immunoreactivity was observed on the microvilli and surface of the receptor cells. These results suggest that immunoreactivity of Gi2α may serve as a sign of functional recovery in the vomeronasal organ following nerve transection.

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261 Poster

IMMUNOHISTOCHEMICAL LOCALIZATION OF G_o IN A SUBSET OF GOLDFISH AND CATFISH OLFACTORY RECEPTOR NEURONS AND BULBAR GLOMERULI

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In rodents, the ciliated receptor neurons (ORNs) of the main olfactory epithelium utilize G protein alpha subunits different than those used by the microvillar ORNs of the vomeronasal organ. The microvillar ORNs use either G_o or G_{i2}, whereas most ciliated ORNs of the main epithelium use G_{olf}. In fish, microvillar and ciliated ORNs are intermingled in the epithelium. However the sensory epithelium is laminar in regards to the expression patterns of identified Buck and Axel type odorant receptors (ORs) and vomeronasal related receptors (V2Rs). Within the sensory region of each lamella the ORNs nearest the surface express V2Rs while the ORNs located in the basal 2/3rds express ORs. We investigated the immunohistochemical localization of the G_o subunit using rabbit polyclonal antisera on cryostat and wholemount preparations of goldfish and catfish olfactory epithelium to test whether this G-protein is preferentially located in one or another ORN type.

In goldfish, the vast majority of positively labeled ORNs were located within the upper half of the epithelium. No immunoreactivity was observed in non-sensory cells. Labeled axons can be traced from the labeled epithelial cells, arching laterally beneath the non-sensory epithelia to collect in the olfactory nerve. The glomerular neuropil exhibits high levels of G_o immunoreactivity. In wholemount preparations of catfish lamellae, labeled ORNs appear localized within the dorsal medial region of the sensory epithelium. The labeled cells are round and are located high in the epithelium, as are microvillar and crypt type ORNs.

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262 Poster

DO AMINO ACIDS STIMULATE CILIATED OR MICROVILLAR OLFACTORY SENSORY NEURONS?

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Fish are capable of detecting a wide variety of odorants using olfactory sensory neurons (OSNs) comprised of mixed populations of ciliated (CRCs) and microvillar receptor cells (MRCs). However, it is unclear if either of these two groups of receptor cells preferentially detect specific classes of odorants. In previous experiments, zebrafish OSNs were labeled in an activity-dependent fashion during stimulation with amino acids (Michel et al. J. Neurosci. Methods 90:143, 1999). In the present study, we use the same technique to identify cell types labeled during exposure to a greater number of amino acids. The AGB control (AGB is itself an odorant) labeled 7% of the sensory epithelium. A binary mixture of AGB and neutral amino acids (L-glutamine, L-methionine or L-alanine) stimulated labeling of 18-21% of sensory epithelium, while binary mixtures of AGB and a basic (L-arginine, 11%) or acidic (L-glutamate, 14%) amino acid stimulated intermediate levels of labeling. It is impossible to unambiguously determine whether the AGB or amino acid stimulated any given OSN in these binary mixture experiments, however, by first identifying the OSN type(s) stimulated by AGB alone it was possible to determine if the binary mixture recruited the other group of OSNs. Since the cell bodies of MRCs are generally more superficial than those of CRCs, we mapped soma locations of labeled OSNs by expressing soma depth as a proportion of total epithelial depth from the apical surface (0) to the basal membrane (1). OSNs stimulated by AGB alone or by binary mixtures displayed overlapping distributions with mean values between 0.3 and 0.4. Thus, the majority of amino acid-stimulated OSNs are located in the superficial half of the epithelium, a zone broadly corresponding to the location of MRCs. An electron microscopy study is currently underway to precisely identify the OSN types stimulated by amino acid odorants.

263 Poster

RESTRICTED DISTRIBUTION AND CHEMOSPECIFICITY OF PHEROMONE-SENSITIVE OLFACTORY RECEPTOR NEURONS IN THE GOLDFISH OLFACTORY EPITHELIUM

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It is not yet established in vertebrates whether socially-relevant stimuli (pheromones) are detected by olfactory receptor neurons (ORNs) different from those that detect food-related stimuli and other odors of general relevance. Goldfish are an excellent model to investigate this question because examples of goldfish sex pheromones and food-related odors are known. Multiunit neural activity was recorded from small populations of ORNs located across the olfactory epithelia of twelve male goldfish to characterize the chemospecificites of ORNs, and to determine whether there is a spatial segregation of ORNs that respond to pheromones and those that respond to food stimuli. Peak integrated (0.5 sec) ORN responses were measured at each of 122 locations while successively exposing fish to: 1) an amino acid (10^{-4} M L-serine, a component of food odor); 2) 10^{-9}M 17,20β-dihydroxy-4-pregnen-3-one (17,20β P, a priming sex pheromone); 3) 10^{-7}M 15-ketoprostaglandin F2a (15K; a releaser sex pheromone); and 4) a bile acid (10^{-7}M taurolithocholic acid sulfate [TLCS], a non-reproductive pheromone). All 122 tested locations contained ORNs that responded to L-serine. Twenty-eight of these 122 positions were also tested with three additional amino acids (10^{-4}M L-arginine, L-methionine, L- glutamic acid) and all were found to respond to all four amino acids. In contrast, of the 65 locations where all four primary odorants were tested, only 20 locations contained ORNs that were responsive to 1720β P, 18 locations were responsive to 15K, and 6 locations to TLCS. There was no tendency for sensitivity to any specific pheromone to be found together with that for any other pheromone, and only eight locations responded to all four stimuli. The majority of the pheromone-sensitive locations were in the dorso-medial regions of the lamellae, locations which Hansen et al. (1999 Microsc. Res. Tech. 45: 325-338) found to contain the highest density of microvillous ORNs.

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264 Poster

ONSET OF CHEMOSENSORY COMPETENCE IN ZEBRAFISH

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The asynchronous expression of ORs and other aspects of the cellular and molecular maturation of the fish olfactory system has been previously described, however corresponding behavioral studies documenting the functional capabilities of the system have not. We have developed an assay to measure the onset and sensitivity of chemosensation in the zebrafish, Danio rerio. Larval fish, occupying 8.0 cm2 chambers, are exposed to odors introduced through a pipette. Sequences of digital images (1 or 2 images per second) are taken before, during and after odorant application. Positive responses are recorded when swimming velocity increases or decreases upon application of water or odorant. Our initial studies focused on the development of sensitivity to the single odorants L-serine (Ser) and taurolithocholic acid (TLCA). Pooled results indicate that fish respond to control water introductions in 13% of trials (n = 23). The proportion of fish responding to TLCA increased with odorant concentration and fish age. Only 25% of animals tested respond to10-7M TLCA at 4 and 5 days post-fertilization (dpf) (n = 4), whereas 67% of 6 dpf animals responded to the same concentration. More fish responded to 10-3M TLCA at 6 dpf then at days 4 or 5 (67% vs. 50%). At 4 dpf, no more than 33% of the fish responded to any concentration of Ser. The peak response to Ser was seen at 5 dpf with 60% of the animals responding to 10-2M Ser (n = 5). At this time, 40% of the animals responded to 10-6 and 10-4 M Ser. Further tests are underway to increase sample sizes and to examine additional ages and odorants. Our preliminary data suggest that those olfactory pathways mediating sensitivity to TLCA and Ser develop early and relatively synchronously.

265 Poster

REGENERATED OLFACTORY ORGAN ENABLES A COMPLETE RECOVERY OF OLFACTORY DISCRIMINATION IN BROWN BULLHEAD CATFISH

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The ability to discriminate the conditioned stimulus, L-Val, from 14 other L-amino acids and 8 binary mixtures before, after, and subsequent to bilateral extirpation and regeneration of the olfactory organ was studied in brown bullhead catfish (*Ameiurus nebulosus*). With the sole exception of L-Ile, L-Val conditioned catfish (n = 15) discriminated the non-conditioned L-amino acids from L-Val. These catfish did not discriminate the binary mixture with L-Val as the more stimulatory component from L-Val, but did discriminate binary mixtures with L-Val as the less stimulatory component from L-Val. Partial regeneration of the olfactory organ was observed two months after extirpation. The same catfish were re-conditioned to L-Val and re-tested. As slopes of regression lines for conditioning and re-conditioning training trials (abscissa - successive conditioning trials, ordinate - fish activity measured as number of >90° turns within 90 secs. of stimulus presentation) were not significantly different, no retention of the conditioned response was detected in animals with regenerated olfactory organs. In the first tests of the re-conditioned animals three months after surgery, the fish did not discriminate L-Met, L-Leu and L-nLeu from L-Val. Although the regenerated olfactory rosette at six months following surgery was still substantially smaller than those of control fish, the discrimination abilities of the catfish with regenerating olfactory organs for amino acids and their binary mixtures were the same as before the surgery. Thus, following regeneration of the olfactory organ in adult catfish, the olfactory system is capable of providing the necessary information to allow the animal to discriminate amino acids by olfaction similar to control catfish.

266 Poster

THE ROLE OF ANTENNULAR SENSILLA IN CHEMO-ORIENTATION OF SPINY LOBSTERS IN A LARGE FLUME.

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Receptor neurons associated with chemosensory sensilla on the antennules of the spiny lobster *Panulirus argus* project to the central nervous system in two parallel pathways. Aesthetasc chemoreceptor neurons project to the olfactory lobes, whereas other chemoreceptor neurons project to the lateral antennular neuropils. How these two pathways function separately and in concert to drive chemically-mediated behaviors remains unclear. We are investigating the roles of each pathway in discrimination of odor quality and orientation with respect to an odor stimulus. The aim of this study was to assess the function of these sensillar pathways for chemo-orientation. Animals were studied in an 8,000-liter flume that generates naturalistic and quantifiable flow conditions. In this study, we used a 5 cm/sec flow rate and low turbulence. To test the importance of aesthetasc sensilla for orientation, we selectively ablated aesthetascs from one group of lobsters and compared their ability to locate an odor source to that of intact animals. Lobsters were released into the flume 2 m downstream from a stimulus source and were allowed to move freely. All trials were videotaped from above, and the two dimensional paths taken by the lobsters were analyzed using motion analysis software. No quantitative differences in path attributes or time to reach the source were identified between intact and aesthetasc-ablated lobsters. These preliminary results suggest that aesthetasc sensilla are not necessary for orientation to an odor source. The sufficiency of aesthetascs for orientation will also be tested by selectively inactivating all non-aesthetasc chemosensory sensilla. The combination of these two studies should reveal the function of each sensillar pathway in orientation.

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267 Poster

THE AESTHETASC PATHWAY IS NOT NECESSARY FOR DISCRIMINATION OF FOOD ODOR MIXTURES BY SPINY LOBSTERS.

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Chemoreceptor neurons (CNs) in the antennules of spiny lobsters (Panulirus argus) innervate different sensillar types that vary in their central projections. CNs in aesthetasc sensilla on the lateral flagellum of the antennules project to the olfactory lobes, large neuropiles with a glomerular organization. By contrast, CNs in non-aesthetasc sensilla on the lateral and medial flagella of the antennules project to the lateral antennular neuropiles (LANs), which are striated and non-glomerular, receive extensive mechanosensory inputs, and are also innervated by antennular motoneurons. To study the roles of different sensillar types in odor discrimination, we have used surgical ablation of specific sensillar types and an aversive conditioning paradigm followed by odor generalization testing. We previously showed that aesthetasc CNs are not necessary for discrimination among 4 complex mixtures that contain partially overlapping sets of 30 - 40 components and that mimic food odors. In the present study, we extend this analysis to examine discrimination of a set of even more similar odors - 4 different blend ratios of a binary mixture, AMP and taurine. The results show that aesthetasc CNs are not necessary for this more challenging discrimination task, suggesting that the non-aesthetasc CNs/LAN pathway is sufficient for these odor discrimination tasks. We are currently investigating the sufficiency of aesthetasc CNs in the same odor discrimination tasks. From the results of other experiments on odor-mediated activation and orientation, we hypothesize that there is some redundancy and interdependence between both antennular chemosensory pathways in spiny lobsters. Supported by NIH DC00312 and The Georgia Research Alliance.

268 Poster

INITIAL STUDIES OF CHEMOSENSORY BEHAVIOR OF MICE DEFICIENT FOR SUBUNIT 1 OF THE CYCLIC NUCLEOTIDE-GATED CHANNEL

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The olfactory cyclic nucleotide-gated (CNG) channel has been postulated to mediate odor-induced depolarizing and hyperpolarizing responses in mouse olfactory receptor neurons (see abstract by Delay and Restrepo). In order to further study the role for this channel in olfaction, we have started to perform behavioral studies in mice defective for subunit 1 of the CNG-channel (CH/KO mice)(Brunet et al., 1996). We have tested naive mice in a retrieval task where the times for recovery of a cracker laced with peanut butter hidden at random locations under the bedding of a cage were recorded. One trial per day was run for five consecutive days. Control offspring from F1 crossings between heterozygous CH/KO females and C57BL/6 males discovered the peanut butter on an average of 300 seconds earlier than a CNG-deficient littermate. Daily search times did not show a consistent decline from day to day. In contrast, the CNG-deficient mice of offspring from F1 crossings of heterozygous CH/KO female mice and FVB males, and control mice found the peanut butter within the same time range. In addition, in contrast to the results with the C57BL/6 offspring, the search time decreased day to day over the five days. The behavioral data indicate that the genetic background may play an important role in chemosensory behavior of CH/KO mice.

269 Poster

OLFACTORY IMPAIRMENT IN HOMOLOGOUS RECOMBINANT MICE DEFICIENT IN THE α SUBUNIT OF G_o.

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Whereas the functions of dendritic G proteins in chemosensory transduction have been well described, little is known about the roles of axonal G proteins, G_o and G_{12}. To begin to investigate the functions of these axonal G proteins we used homologous recombinant mice deficient in the α subunit of G_o, graciously provided by Dr. Eva Neer. The original 129/C57Bl6 mice yielded less viable G_o-/- offspring than expected due to inbreeding depression. Intercrossing F1 mice after a single generation of backcross into the outbred CD1 genetic background increased viability of G_o-/- offspring. Although smaller in weight than their heterozygous litter mates, these mice did not show obvious behavioral abnormalities, as reported previously, and their life span was markedly increased from that reported in the inbred genetic background. Whereas all of the heterozygotes were able to readily locate a buried food pellet after an overnight starvation period, none of the G_o-/- mice succeeded in locating the pellet within the 4 min assay period. Olfactory ability of the G_o-/- mice was further assessed by an olfactory habituation/dishabituation test. When control heterozygous mice are presented with an unfamiliar odor on a cotton wool swab protruding from the cage lid, they rear up to investigate the odor. The number of rearings and total rearing time abates as they habituate to the odor, but can be elicited *de novo* by introducing a different odor. The number of rearings in response to sequential odor exposures was markedly lower or virtually absent in G_o-/- mice, although their mobility was not impaired. We conclude that absence of the a subunit of G_o compromises olfactory ability. The impact of deletion of G_o on the formation of chemotopic projections and/or compensatory expression of other G proteins is currently under investigation.

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270 Poster

OLFACTORY DISCRIMINATION PERFORMANCE IN MONKEYS, HUMANS AND HONEYBEES: MAMMALS AND INSECTS SHARE COMMON PRINCIPLES OF ODOR QUALITY PERCEPTION

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Animals of most species are capable of discriminating between a variety of odors. To understand the meachanisms underlying odor discrimination it is necessary to establish which properties of an odor molecule are functional in determining the degree of interaction with a given receptor, and thus in determining its perceived odor quality. One useful means to assess possible correlations between odor quality and molecular properties is to test the discriminability of structurally related odorants.

In a first series of studies, we tested the ability of human subjects to distinguish between members of five series of aliphatic substances and compared their performance with that of squirrel monkeys and honeybees. With all substance classes, and in all three species, we found a significant negative correlation between discrimination performance and structural similarity of odorants in terms of differences in carbon chain length. Further, in all three species we found both position and type of oxygen moiety to affect discriminability in a substance class-specific manner.

In a second series of studies, we tested the ability of human subjects to distinguish between ten pairs of enantiomers and again we compared their performance with that of squirrel monkeys and honeybees. We found that all three species were able to significantly discriminate between the enantiomers of alpha-pinene, limonene and carvone, whereas they failed to distinguish between the (+)- and (-)-forms of alpha-terpineol, camphor, rose oxide and 2-butanol, thus showing very similar patterns of discrimination performance.

Taken together, the results of these studies provide evidence of striking parallels in olfactory discrimination abilities between primates and honeybees.

Thus, our findings support the assumptions that mammals and insects may share common principles of odor quality perception, irrespective of their completely differing repertoires of olfactory receptors, and that in both taxa enantioselective receptors may only exist for some but not all volatile enatiomers.

271 Poster

USE OF "ELECTRIC ODORS" AS A DISCRIMINATIVE CUE: COMPARISON OF STIMULATION OF THE LATERAL OLFACTORY TRACT AND PIRIFORM CORTEX ASSOCIATION FIBERS

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Previous research has demonstrated that patterned electrical stimulation of the olfactory bulb (Mouly et al., Behav. Brain Res. 17: 45-58, 1985) or of its output pathway, the lateral olfactory tract (LOT) (Roman et al., Brain Res. 418: 221-226, 1987), can be used as a discriminative cue in a learning task (so-called "electric odors"). Because olfactory bulb stimulation activates association fibers from piriform cortex as well as the LOT, we compared the effectiveness of stimulation of these two fiber systems as a discriminative cue. Male Long-Evans rats with chronically implanted electrodes were water deprived and trained on a go/no-go task in which they initiated a trial by breaking a photobeam in a nose-poke operandum. Animals received a water reward for performing a second nose-poke on "go" trials in which the first nose poke triggered patterned electrical stimulation (a single train of 4 pulses at 40 Hz), and for withholding a response on "no-go" trials in which no stimulation was given. All animals attained high levels of performance during initial training, which used stimulation that activated both the LOT and cortical association fibers (coactivation). Animals were then tested for their ability to respond to stimulation that selectively activated each fiber system on different trials. Stimulation of cortical association fibers was found to be significantly more effective as a discriminative cue that was LOT stimulation. In a final test, stimulation of cortical association fibers was found to be effective as a discriminative cue even if the number of pulses in an "electric odor" was reduced from 4 to 1. These results indicate that activation of cortical association fibers plays a critical role in the effectiveness of "electric odors," and emphasize the importance of this fiber system in olfactory learning. Supported by NIH grant DC02271 (NIDCD).

272 Poster

MHC-DETERMINED ODORTYPES MODULATE MOTHER-OFFSPRING RELATIONS IN MICE.

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Studies in several different species have shown that a mother can discriminate her own offspring from alien offspring by odor and offspring can recognize maternal odors but no specific genetic basis for this has yet been identified. We and others have shown that the Major Histocompatibility Complex (MHC) of genes, a hypervariable set of linked genes known to be involved in immune rejection, is also involved in determining an animal'fs genetically-determined olfactory identity (an animals odortype). Previous studies demonstrating that MHC-odortypes are evident in mice as young as 1 day raised the possibility that MHC odortypes could underlie mother-infant recognition and discrimination. The purpose of these studies was to determine (a) whether mothers respond differentially toward pups according to the pup MHC type (b) whether offspring respond differentially to littermates and/or lactating females on the basis of their MHC odortypes and (c) whether postnatal experience modulates pup preference. Females preferentially retrieve infants identical to their own MHC compared to infants differing from themselves and from their infants at the MHC. Reciprocally, infants preferentially approach nest-derived odors produced by individuals of the same MHC type as their mothers and littermates compared to nest odors from MHC-dissimilar mothers and litters. Early experiences influence the expression of some of these preferences.

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273 Poster

COMPARING BRIEF-ACCESS TASTE TESTS TO PREFERENCE TESTS IN INBRED AND CONGENIC MICE

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Inbred mice vary in their response to bitter-tasting compounds as assessed by 48-h preference tests. These differences are generally assumed to result from altered gustatory function, although such long-term tests could easily reflect additional factors. We developed a brief-access taste test for mice and compared the results of preference tests to the brief tests over a consistent concentration range for the bitter-tasting substance sucrose octaacetate (SOA). Water-deprived mice were trained in a modified Davis apparatus to lick water from a stainless steel spout. In the testing, mice were presented with five concentrations of SOA (0.00018 - 0.18 mM) and distilled water. Trials were 5 s in duration and stimuli were presented randomly within blocks; each stimulus trial was preceded by a water trial. Each concentration was presented twice in a session and mice were repeatedly tested across consecutive days. SOA-taster mice, including the SWR/J (SW) inbred and the C3.SW-*Soa*^{a} congenic strains, avoided licking SOA at concentrations greater than 0.003 mM. C3HeB/FeJ (C3) inbred mice licked all concentrations at the same rate as water. Concentration-response functions were similar for both the brief-access test and a parallel 48-hr preference test run on separate groups of mice. These results confirm that SOA aversion is mediated by a gustatory cue. We then used our brief-access test procedure to examine the responses of SW and C3 mice to a variety of bitter and non-bitter stimuli. In comparison to the C3 mice, the SW strain was more sensitive to the taste of quinine-HCl, caffeine, and Na-saccharin; there were no differences for citric acid or NaCl. We conclude that this brief access procedure is an effective method to study gustatory strain differences in mice.

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274 Poster

SODIUM CHLORIDE TASTE DETECTION PERFORMANCE OF C57BL/6J MICE IN AN OPERANT CONDITIONING PARADIGM

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Inbred mice have been increasingly used as a model for taste research owing to their usefulness in genetic experimentation. The most common behavioral method used to assess taste responsiveness in mice has been the long-term intake test. Although informative as a preliminary assay of taste function, this method has potential for postingestive events to influence the amount consumed. Moreover, this test relies on inherent hedonic characteristics of the chemical stimuli to drive the behavior. To better understand NaCl taste sensitivity in mice, we used a two-response operant discrimination procedure, where the taste of the solution served as a signal for reinforcement. Ten C57BL/6J mice, on a restricted water-access schedule, were trained, in a specially designed gustometer, to lick from a centrally positioned sample spout (5 licks) and respond by licking from one reinforcement spout (left or right, counterbalanced between animals) in response to 0.6 M NaCl and from the opposite spout in response to water. Correct responses were reinforced with 15 licks of water. Of the 10 mice initially trained, 7 were tested with water and an array of NaCl concentrations (0.2, 0.4, 0.6 M) using the same procedure. By the third week percentage correct responses reached 66%, 76%, and 84% for these concentrations, respectively. The false alarm rate for water was 18%, a value that significantly differed from each NaCl concentration. We are in the process of deriving complete psychometric functions describing NaCl sensitivity in these mice. Our procedure, in which small fluid volumes are delivered and immediate responses are measured, reduces the possibility of postingestive effects guiding the behavior and separates the discriminative from the hedonic properties of the stimulus. This procedure shows great promise for taste research in light of the rapid developments in mapping and manipulating the mouse genome.

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275 Poster

DISCRIMINATION BETWEEN SUCROSE TASTE AND MONOSODIUM GLUTAMATE TASTE IN RATS.

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Monosodium glutamate (MSG), found in meats, fish and other foods, elicits a taste called *umami*. Sucrose is a prototypic sweetener. However, under certain conditions, sucrose mimics the taste of MSG in rats. For example, if a taste aversion is conditioned to MSG mixed with amiloride, the aversion generalizes to sucrose. Amiloride, tasteless in small quantities, is a cation channel blocker that is believed to reduce the contribution of Na^+ to MSG taste. In several species, stimulating the tongue with MSG excites sucrose-best fibers (S-fibers) and NaCl-best fibers (N fibers). Collectively, these data suggest that some aspect of the taste of MSG is similar to sucrose and raise the question of whether transduction pathways of MSG and sucrose interact. We conducted several experiments using a shock-avoidance discrimination method to determine how well rats can discriminate the taste of sucrose from that of MSG mixed with amiloride. The first experiment determined that the threshold of MSG (1-2 mM) was similar to that of sucrose (3-4 mM) when amiloride (50 μ M) was mixed in all solutions. In the second experiment, rats were tested with a range of concentrations (2.5-100 mM) of MSG and sucrose (all containing 50 μ M amiloride). The results suggest that rats can discriminate between sucrose and MSG at concentrations above 20 mM. Discriminability between these stimuli decreased near detection thresholds. In the third experiment, equimolar concentrations of NaCl were added to sucrose stimuli. NaCl did not alter discriminability between MSG and sucrose when amiloride (50 m M) was present. Thus, although taste aversion studies suggest that rats perceive the taste of MSG with amiloride as similar to sucrose, discrimination methods show that rats can discriminate between these stimuli. Experiments underway are testing discrimination between these substances in the absence of amiloride.

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276 Poster

CORN OIL/MINERAL OIL DISCRIMINATION BY THE RAT

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We have previously shown that when rats are given a LiCl injection following ingestion of a sucrose/corn oil mixture they reject this mixture in subsequent preference tests. Furthermore, this taste aversion generalizes to the corn oil, but not to the sucrose component of the mixture. Because these findings have been demonstrated in short -term preference tests, we have concluded that this conditioned flavor aversion is the result of orosensory, but not, post ingestional factors. The current experiments were designed to explore the role of textural cues in this flavor conditioning. Rats were given LiCl injections following ingestion of a sucrose/corn oil mixture and then subjected to preference tests between sucrose/corn oil and sucrose/mineral oil mixtures. These preference tests were conducted in three ways:

1. One-hour preference tests were conducted daily between the two sucrose/oil mixtures for 8 days following conditioning. 2. For a second group of rats, similar one-hr preference tests were run in a special cage where the actual number of licks on each tube could be measured every six seconds. 3. Aversion to the solutions were measured in 30 s single-bottle presentations in a Davis Apparatus, where ingestive behavior to multiple stimulus presentations could be measured over a five minute period with 1 ms resolution. The latter two methods allowed for study of the formation of the aversion over time. The results from these measure show that the rats can easily discriminate between the two oils and that the discrimination occurs within the first two minutes of the tests. Assuming that the viscosity of corn and mineral oil is similar, these data minimize the possibility that texture plays a major role in this rapid discrimination. Further tests were conducted to assess the roles of olfaction and of gustation in the discrimination of the oil mixtures.

277 Poster

SHORT-TERM TASTE SPECIFICITY IN CONDITIONED TASTE AVERSION

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Rats can express a conditioned taste aversion (CTA) within 1h of receiving a toxin paired with a novel tastant. To determine if this short-term expression of CTA is taste specific, the specificity of a sucrose CTA was tested by measuring the intraoral intake of sucrose or NaCl 1h or 48h after pairing sucrose and LiCl.

Rats (n = 3-7/group) were surgically implanted with intraoral catheters and received three daily infusions of water. Rats received an intraoral infusion of 5% sucrose (6ml/6min). Thirty minutes later, they were injected with LiCl (0.15M,12ml/kg i.p.). Sucrose or 0.45% NaCl was then infused 1h or 48h after LiCl. To control for toxic effects, rats were given LiCl with no prior access to sucrose and infused at 1h and 48h with sucrose or NaCl.

Prior to LiCl injection, rats consumed 5.22±0.23g of sucrose. One hour following a sucrose-LiCl pairing, rats rejected both sucrose (0.0±0.2g) and NaCl (1.1±0.8g) infusions (n.s.). Forty-eight hours following the pairing, rats rejected sucrose (2.52 ± 0.46g) but consumed NaCl (5.47±0.23g; $p < 0.01$). The rejection of NaCl or sucrose at 1h was not due to toxic effects of the LiCl because rats receiving LiCl without prior sucrose consumed both sucrose (4.4 ± 0.65g) and NaCl (4.7 ± 1.2g) 1h after the LiCl injection.

We conclude that because rats rejected both sucrose and NaCl 1h after the pairing, the short-term CTA is not taste specific at 1h. Because rats rejected sucrose but consumed NaCl 48h after the pairing, the CTA must become taste specific within 48h.

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278 Poster

THE GENERALIZATION OF TASTE AVERSIONS TO MIXTURES OF SUCROSE, SODIUM CHLORIDE, AND QUININE.HCL IN HAMSTERS

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During food sampling, the gustatory system often evaluates taste mixtures that contain stimuli with different taste qualities. In the hamster (*Mesocricetus auratus*) the integration of signals from these heterogeneous taste mixtures begins in the gustatory periphery (Formaker and Frank, 1996; Formaker et al., 1997). In a concentration dependent manner, quinine.HCl (QHCl) inhibits chorda tympani (CT) neural responses to sucrose and NaCl inhibits responses to QHCl. In order to examine the behavioral consequences of these peripheral gustatory interactions, we trained 7 groups of 4 hamsters to each avoid one conditioning stimulus (CS). The CSs were 100mM NaCl, 100mM sucrose, 1mM QHCl, and the 3 binary and 1 ternary mixtures of those stimuli. The CS for the control group (n = 8) was deionized water. Testing was conducted with all 8 stimuli that served as CSs. Each animal underwent two conditioning trials and two cycles of testing. In general, binary mixtures and mixture components cross-generalized, with one notable exception. QHCl was not recognizable when mixed with NaCl. Animals conditioned to avoid a mixture containing NaCl and QHCl suppressed their intake of NaCl, but not QHCl. Furthermore, animals conditioned to avoid NaCl suppressed their intake of the NaCl-QHCl mixture, but animals conditioned to avoid QHCl did not. The aversion to the ternary mixture generalized to all test stimuli except QHCl and water. No CS generalized to water. We conclude that NaCl masks the taste of QHCl at the concentrations tested, but QHCl does not mask the taste of sucrose. The previously reported inhibition of CT sucrose responses by QHCl likely reflects receptor events. However, the suppressive effects of QHCl on neural responses to sucrose are greater at concentrations exceeding 1mM. Because hamsters naturally avoid QHCl, behavioral correlates of these neural interactions may be difficult to demonstrate at higher concentrations.

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279 Poster

BILATERAL LESIONS OF THE PARABRACHIAL NUCLEUS REVERSE THE NACL AVERSION OF FISCHER-344 RATS.

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Fischer-344 (F-344) rats do not display a preference for NaCl solutions compared to water, and exhibit aversions to NaCl at concentrations usually preferred by other strains of rats. Evidence that chorda tympani, but not glossopharyngeal, transections reverse this aversion suggests that signals conveyed by the chorda tympani play a critical role in F-344 NaCl aversion. It is not known, however, whether lesions within the central gustatory pathway alter the NaCl aversion of F-344 rats. To begin to address this question, we lesioned the parabrachial nucleus (PBN) since it is an obligatory synapse for ascending taste information. Sodium intake was measured in bilateral PBN-lesioned (PBNX, n = 17) and sham-lesioned (n = 14) F-344 rats, using the two-bottle preference paradigm. Rats were given access to 0.15M NaCl and distilled water and intake of each solution was measured every 24h for 4 days. Statistical analyses revealed that PBNX animals exhibited a significant increase in salt preference ratios, relative to the F-344 sham-lesioned subjects. Thus, when the PBN is lesioned, F-344 rats do not display NaCl aversion, but instead, display NaCl preference similar to Wistar rats, a NaCl preferring strain. These results are strikingly similar to the effects reported following chorda tympani transections. We suggest that PBN lesions affect NaCl aversion in F-344 rats, either by disrupting the processing of NaCl information within this nucleus, or by interrupting ascending NaCl information en route to forebrain structures.

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280 Poster

THE ROLE OF SUBSTANCE-P IN SIGNALING THE PRESENCE OF THE ORAL IRRITANT CAPSAICIN.

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The brainstem trigeminal complex, and particularly subnucleus caudalis (Vc), processes nociceptive information originating in the orofacial region including irritation elicited by chemicals such as capsaicin. Substance P (SP) is a neuropeptide that functions as a neurotransmitter/modulator in trigeminal nociceptive afferents projecting to Vc. Recently, knockout (KO) mice have been developed in which the gene encoding preprotachykinins has been deleted, resulting in an absence of SP and neurokinin A. We tested the hypothesis that mice lacking SP have a deficit in detecting irritant chemicals. A paired preference taste paradigm was used to assess discrimination between water and various concentrations of capsaicin (0, 0.1, 0.25, 1, 2 and 5 ppm) by the KO mice, with wildtype (WT) mice serving as a control group. Fluid consumption was determined on alternate days by weighing the bottles, and then switching their position to control for positional preference. At suprathreshold capsaicin concentrations (2 ppm or higher), both the WT and KO showed a significant ($p < 0.001$) concentration-dependent decrease in consumption of the capsaicin solution. However, at threshold concentrations the KO exhibited less aversion to the capsaicin. Thus, KO consumption of 1 ppm capsaicin was not significantly different from water, while WT consumed significantly ($p < 0.05$) less capsaicin at 1 ppm. These results indicate that SP has a role in the detection of near-threshold irritant chemicals contacting the oral cavity. However, since the KO showed clear aversion to suprathreshold concentrations of capsaicin, other neurotransmitters such as glutamate must play an integral role in signaling oral irritation.

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281 Slide

SYSTEMATIC DIFFERENCES IN GLOMERULAR RESPONSES TO ORGANIC ACID ODORANTS POSSESSING DISTINCT HYDROCARBON STRUCTURES

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Straight-chained aliphatic acid odorants of different carbon number produce systematically different spatial patterns of 2-deoxyglucose uptake in the glomerular layer of the olfactory bulb (Johnson et al., 1999, J. Comp. Neurol. 409:529-548). One of the effects of increasing carbon number was a ventral shift in the location of the anterior, dorsomedial response known to be evoked by numerous organic acids. Since increasing carbon number is correlated with progressive increases in several specific molecular properties, including hydrophobicity, molecular volume, and molecular length, we wanted to further explore which of these chemical properties are most associated with the location of the dorsomedial representation in the bulb. Therefore, we exposed rats to either five- and six-carbon organic acids of distinct hydrocarbon structure, including straight-chained, branched, cyclic, and double-bonded molecules. We found that odorant molecular length is the property that most strongly correlates with the dorsal-ventral position of the dorsomedial response ($r = 0.85$, $p = 0.002$). The exquisite relationship between spatial locations of response and a specific molecular property of these odorants suggests that the olfactory bulb may perform a sophisticated molecular feature analysis through spatial arrangements of individual glomeruli within a larger glomerular response module. The distinct hydrocarbon structures also produced large differences in the spatial patterns of 2-deoxyglucose uptake in the posterior, lateral and posterior, medial regions of the bulb. Among the tested odorants, 2-methylbutyric acid was remarkable for evoking robust activity in a large cluster of glomeruli organized along the rostral-caudal axis. This was in contrast to the structurally similar odorant, 3-methylbutyric (isovaleric) acid, which only weakly stimulated the same region. These data suggest that the posterior portions of the olfactory bulb may encode specific steric features of the odorant molecules, and that some odorants may have an intrinsically greater bulbar representation than others.

282 Slide

SPATIAL PATTERNS OF OLFACTORY RECEPTOR NEURON INPUT TO TURTLE OLFACTORY BULB GLOMERULI IMAGED WITH CALCIUM-SENSITIVE DYES

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We used a protocol adapted from Friedrich and Korsching (Neuron 18:737-752) to selectively label turtle olfactory receptor neurons with Calcium Green dextran and image patterns of odor-evoked input to the olfactory bulb. We used a wide-field imaging system and a high frame-rate CCD camera to image activity across the entire dorsal bulb with a temporal resolution of up to 1 kHz and minimal or no signal averaging. In each preparation, odor-evoked activity was recorded in response to several (4 - 6) single odors over a concentration range of up to two log units. The temporal character of the odor-evoked signals was simple, with a smooth rise and slow decay, and similar for all odors and locations. Different odors evoked distinct, but often overlapping, spatial patterns of activity with several well-defined peaks encompassing areas estimated to include at least a dozen glomeruli. At the same time, most odors also evoked more widespread regions of moderate activity, which for some odors included up to 60% of the dorsal surface. Regions activated by a given odor were similar across animals and bilaterally symmetrical. The relative pattern of activity evoked by an odor was remarkably consistent across all concentrations tested. However, the absolute magnitude of the odor-evoked signals increased with concentration, with the result that, at higher odor concentrations, odors evoked significant levels of activity across a large fraction of the dorsal bulb surface, indicating activation of many different receptor neuron populations. These findings appear to reflect a relatively broad tuning of olfactory receptor neurons, and suggest that higher-order olfactory processing must involve recognizing the relative pattern of input across many glomeruli, as opposed to simply detecting whether a particular glomerulus or set of glomeruli is activated above a given threshold.

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283 Slide

PERIPHERAL OLFACTORY PROJECTIONS ARE DIFFERENTIALLY AFFECTED IN MICE DEFICIENT IN A CYCLIC NUCLEOTIDE-GATED CHANNEL SUBUNIT

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Axons of olfactory sensory neurons expressing a given odorant receptor converge to a few glomeruli in the olfactory bulb. We have generated mice with unresponsive olfactory sensory neurons by targeted mutagenesis of a cyclic nucleotide-gated channel subunit gene, OCNC1. When these anosmic mice were crossed with mice in which neurons expressing a given odorant receptor can be visualized by co-expression of an axonal marker, the pattern of convergence was affected for one but not another receptor. In a novel paradigm, termed monoallelic deprivation, axons from channel positive or negative neurons that express the same odorant receptor segregate into distinct glomeruli within the same bulb. Thus, the peripheral olfactory projections are in part influenced by mechanisms that depend on neuronal activity.

284 Slide

ODOR ELICITED ACTIVITY PATTERNS IN RAT MAIN OLFACTORY BULB MAPPED BY FUNCTIONAL MAGNETIC RESONANCE IMAGING

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Functional magnetic resonance imaging (fMRI) has been introduced to map the spatial patterns elicited by odor in the MOB (Yang et al, PNAS, 95:7155). To optimize the fMRI method for further analysis, we have increased the mapping from single MOB slices to 20 slices comprising the entire MOB and improved the spatial resolution from 220x220x1000 um to 220x220x250 um and temporal resolution from 8s to 1.5s/image. We used these new parameters to reveal the activity patterns (APs) elicited by odorants and to study the effect of odorant concentration on the APs. Correlation of the anatomical image obtained from MRI with the histological layers of the MOB showed that the activities in individual slices were mainly located at the glomerular layer, in correspondence with the highest capillary density there and the highest energy consumption by 2-deoxyglucose (2-DG) mapping. Across the whole bulb, APs had the same general features: (A). Stronger activities in the lateral regions of the anterior MOB slices and at the medial regions of the posterior slices; and (B). Connected activity foci instead of isolated random 'hot spots' in the adjacent slices. These results are in agreement with the projection pattern of ORNs and the activity patterns in the MOB demonstrated by other methods. Different odorants elicited overlapping but different APs, suggesting that although individual glomeruli could be activated by different odors, the APs that were associated with the activity of all glomeruli are specific (even for optical isomers, such as carvones). With increased odorant concentration, both the area and the activity of the focus were increased. However, the APs were almost identical topographically, indicating that the AP contains intrinsic properties of an olfactory stimulus, such as quality and quantity information of an odor.

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285 Slide

TWO-PHOTON MICROSCOPY OF THE DEVELOPING OLFACTORY SYSTEM

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The first step in olfactory perception resides in the interaction of odorous molecules with odorant receptors (ORs) on the surface of olfactory sensory neurons (OSNs). OR genes represent a large multigene family encoding seven-transmembrane proteins. OSNs that express a given OR project their axons to a pair of glomeruli in the olfactory bulb that are located at recognizable positions. Here, we have applied gene-targeting techniques to construct strains of mice in which all OSNs or those that express a particular OR gene, while they are alive, can be visualized by virtue of their coexpression of the green fluorescent protein (GFP). Utilizing two-photon microscopy we imaged the axonal projections of OSNs and uncovered a high degree of morphological variability among genetically defined mature glomeruli. A protoglomerulus coalesces from a tangle of converging fibers at a reproducible day early postnatally. Videos will be shown with animations of three-dimensional reconstructions of glomeruli at various developmental stages. Our genetically based imaging approach should be generally applicable in neurobiology.

286 Slide

A NOVEL METHOD FOR RAPID SCREENING OF PUTATIVE ACTIVATORS OF VOMERONASAL RECEPTOR NEURONS.

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The vomeronasal organ (VNO) has been implicated in the detection of chemical cues, broadly defined as pheromones, which appear to evoke innate hormonal and behavioral responses in terrestrial vertebrates. Pheromones are thought to induce their effects by activating vomeronasal receptors and a G-protein coupled second messenger cascade. Although in rodents urine is believed to be the major source of pheromones, the number of identified urinary pheromones is very limited. In patch-clamp experiments vomeronasal receptor neurons (VRNs) have been shown to respond after the application of urine and urine-derived compounds, but they failed to respond to pharmacological compounds designed to activate components of a second-messenger pathway.

To better understand the mechanism of vomeronasal neuron transduction it would be useful to extend the number of substances that activate VRNs. Neither behavioral experiments nor patch-clamp recording is suitable for the rapid identification of putative pheromones or other activating substances. To screen for a large number of compounds that could be potential ligands, or that could activate second messenger pathway(s) we have developed a whole-nerve VNO preparation. VRN axons collect into a small number of nerve bundles, from which the total response of all neurons sending their axons to a defined group of nerves can be recorded using extracellular electrodes. Perfusion with KCl (2 to 10 mM) induced reversible depolarizations that could be repeated even after 12 hours, indicating long lasting viability of the isolated whole-nerve preparation. We were able to identify a large number of non-pheromonal compounds that activated VNRs. These substances include activators of second messengers and compounds that might react with vomeronasal receptors.

In conclusion, the whole-nerve VNO preparation is suitable for the rapid screening of compounds that might activate VRNs.

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287 Slide

MOUSE VOMERONASAL NEURONS ARE HIGHLY SELECTIVE AND ULTRASENSITIVE PHEROMONE DETECTORS

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The vomeronasal organ (VNO) is a chemoreceptive structure thought to be specialized for the transduction of pheromones into electrical responses that regulate sexual, hormonal, and reproductive function in many mammals. However, it has been very difficult to measure stimulus-induced responses in neurons of the VNO. Therefore, it is currently not known which stimuli are detected by vomeronasal neurons (VNs) or how sensory processing is achieved at the cellular level. We have developed a mouse VNO slice preparation to fill this gap in our knowledge and demonstrate for the first time that six biologically relevant, putative pheromones evoke excitatory responses in single VNs leading to action potential generation and elevated Ca2+ entry. The detection threshold for some of these chemicals is remarkably low, placing VNs among the most sensitive chemodetectors in mammals described so far. Using confocal Ca2+ imaging, we map the epithelial representation of the putative pheromones to show that each of the ligands activates a unique, nonoverlapping subset of VNs. All VNs responding to a given putative pheromone exhibited highly selective tuning properties. These results indicate that, in contrast to the main olfactory epithelium, the VNO uses a noncombinatorial coding scheme for processing of chemosensory information. Our results establish mouse vomeronasal neurons as highly selective and ultrasensitive pheromone detectors and provide a basis for understanding neural processing of chemical signals that regulate mammalian communication and sexual behavior.

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288 Slide

ADENOSINE-CYCLIC MONO PHOSPHATE SIGNALLING IN RAT VOMERONASAL ORGAN: ROLE OF ADENYLYL CYCLASE SUBTYPE VI

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Adenosine-cyclic mono phosphate signalling in rat vomeronasal organ: role of an adenylyl cyclase type VI

Chemosensory neurons in the vomeronasal organ (VNO) detect pheromones related to social and reproductive behavior in most terrestrial vertebrates. The chemoelectrical transduction process in the VNO is mediated by G protein-coupled second messenger cascades. The results of the present study demonstrate that stimulation of female rat vomeronasal organ microvillar preparations with male rat urine not only induce a rapid and transient IP3 signal, but in addition, the level of cAMP decreases with a delayed and sustained time course. The decrease in cAMP seems to be a consequence of the preceding activation of the phosphoinositol pathway rather than the result of an enhanced phosphodiesterase activity or an inhibition of adenylyl cyclase via Gαi or Gα o. This notion is supported by the finding that activation of the endogenous protein kinase C suppresses basal as well as forskolin-induced cAMP-formation; furthermore, elevated levels of calcium inhibit cAMP-formation in rat VNO microvillar preparations. These properties of cAMP-signalling in the VNO of rats may be mediated by an adenylyl cyclase subtype VI, which is localized in microvillar preparations of the VNO.

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289 Slide

SEXUAL DIMORPHISM AND DEVELOPMENTAL EXPRESSION OF SIGNAL TRANSDUCTION MACHINERY IN THE VOMERONASAL ORGAN

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We have developed a new model to study the transduction of chemosignals in the VNO, for which the functional pathway for chemical communication is incompletely understood. *Sternotherus odoratus* (musk turtle) has a proportionally larger VNO than mammals, vomeronasal sensory neurons (VSNs) can be isolated for patch-clamp recordings, and species-specific chemosignal (musk) can be harvested. The turtle vomeronasal epithelium (VNE) was found to contain the G-proteins G_{β} and $G_{\alpha\ il\text{-}3}$ at the microvilli, as evidenced by immunocytochemical techniques. $G_{\alpha\ o}$ labeled the axon bundles in the VNE and the somata of the VSNs and not the microvilli. Densitometric analysis of Western blots indicated that VNO from females contains a greater concentration of $G_{\alpha\ il\text{-}3}$ than that of males. Sexually immature (juvenile) turtles show intense immunolabeling for all three subunits (G_{β}, $G_{\alpha\ il\text{-}3}$, $G_{\alpha\ o}$) in the axon bundles and an absence of labeling of the microvilli. Transient receptor potential channel (TRP2) was visualized by Western blot in rat and turtle VNO and is more highly expressed in males than in females in both species. Postnatal expression of TRP2 in rats incrementally increases through day 20, where protein expression is equivalent to the adult. Stimulation of turtle VSNs with depolarizing voltage-steps evokes an outward-transient current that is approximately 25% greater in females than in males. In behavioral paradigms where turtles were presented with 3 choices, musk (0.3 ppm), own tank water (300 ppm), or control water, male turtles spent equal time in each area when testing male musk, but spent twice as long in the musk area when testing female musk. These data demonstrate the utility of *Sternotherus* for discerning the functional signal transduction machinery in the VNO and suggests that gender and developmental differences in effector proteins or cellular-signaling components may be used to activate sex-specific behaviors.

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290 Slide

VNO-RELATED RECEPTORS IN ZEBRAFISH

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In mammals, odorants are detected in the nasal olfactory epithelium (OE) whereas pheromones are thought to be detected primarily in the vomeronasal organ (VNO). In fish, which do not have a VNO, both odorants and pheromones are detected in the olfactory epithelium of the olfactory rosette. Three families of olfactory receptors have been identified in mammals: the odorant receptor (OR) family, with ~1000 members, and two smaller families of VNO receptors, the VNRs (~35 members) and the VRs (~140 members). Families of ORs homologous to those in mammals have been identified in all vertebrate species examined, including fish. Surprisingly, VR-like receptors have also been identified in fish, but here they are expressed in the OE. In our initial studies, we amplified VR-related genes from the zebrafish genome and found that they were expressed in OE neurons. Sequence analyses of zebrafish VR (zVR) cDNA clones showed that zVRs are distinct from, but resemble, mouse VRs. From genomic library screens, we estimate that there are ~45 VR genes and ~45-50 OR genes in zebrafish. As in goldfish (Cao et al, 1998), in situ hybridization with zVR and zOR clones revealed that the two receptor types are expressed in different OE zones that are reminiscent of those seen in the mouse VNO. To gain insight into the respective functions of zVRs and zORs, we are using a combination of calcium imaging and single cell RT-PCR to identify receptors that recognize chemicals known to stimulate fish OE neurons. A goldfish VR-like receptor was recently shown to recognize arginine and lysine (Speca et al, 1999). In our studies, we found that two neurons that each responded to one amino acid (tryptophan or cysteine), but not other amino acids, bile acids, or putative pheromones, each expressed a zVR gene.

291 Poster

EFFECT OF DIAZEPAM ON INHIBITORY POSTSYNAPTIC POTENTIALS IN NUCLEUS OF THE SOLITARY TRACT OF NEONATAL RATS IS TEMPERATURE DEPENDENT

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Injection of the benzodiazepine (BZ) receptor agonist diazepam (Valium) into the 4th ventricle enhances food palatibility and intake (Berridge and Peciña, Neurosci, Biobehav.Rev. 19:121-131, 1995) and it has been hypothesized that this phenomenon results from the action of diazepam on neurons in the nucleus of the solitary tract (NST). In other brain areas benzodiazepines facilitate GABA-induced inhibitory potentials (IPSP) in postsynaptic neurons that have GABAA receptors, by modulating Cl- channel activity. Recent data indicates that GABA-ergic synaptic transmission is important in processing gustatory information in the NTS (Grabaukas and Bradley, Neuroscience 94:1173-1182) and therefore diazepam may exert its effect on palatibility by enhancing inhibitory activity in NTS neurons. We have used whole-cell patch-clamp recording in horizontal brainstem slices of neonatal rats (P0-10) maintained at room temperature (21°C) to determine if NST neurons posses GABAA receptors with BZ binding sites. Pharmacologically isolated IPSPs were evoked by single-shock electrical stimulation of GABA-ergic neurons in the presence of glutamate receptor blockers (CNQX and APV). Diazepam (1 and 4mM) was superfused over the slices. Under control conditions the mean decay time constant of an IPSP was 139ms±17 and all NTS neurons were insensitive to diazepam suggesting that NTS GABA receptors do not express BZ receptors. However, the decay time was temperature sensitive with a coefficient of dependency (Q10) of ~ 2.3 and application of diazepam at 36°C prolonged the decay time of the IPSP in a concentration dependent manner. Thus, at normal body temperature NTS neurons express GABAA receptors with BZ binding sites. It is possible, therefore, that application of BZ into the 4th ventricle influences food intake by potentiation of GABA-ergic neurotransmission in the NST.

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292 Poster

$GABA_A$ AND $GABA_B$ ANTAGONISTS ALTER EVOKED FIELD POTENTIALS IN SENSORY LAYERS OF GOLDFISH GUSTATORY LOBES.

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The vagal lobes of the goldfish are large, laminated structures homologous to the gustatory portion of the mammalian nucleus of the solitary tract. Field potentials can be evoked in the sensory layers of vagal lobe slices by electrically stimulating the afferent fibers (Finger and Dunwiddie, Brain Res. 580:27-34, 1992). These potentials typically consist of two or three rapid, negative going potentials (N1-3) which are sometimes followed by a rapid, positive going potential. Presentation of a second stimulus within 100 ms of the first typically results in the decrease of N2 and an increase of N3 and the positive going potential. The principal excitatory components of these potentials (N2+3) are mediated by ionotropic glutamate receptors (Smeraski et al., Chem. Senses 24:37-46, 1999), but the inhibitory components of this response have not been characterized.

We have examined the effects of bath application of the $GABA_A$ and $GABA_B$ antagonists bicuculline and CGP 55845 on field potentials in vagal lobe slices. Bicuculline typically has no effect on N1 or N2, but causes an enhancement of N3 and increases the latency and duration of the positive going potential. Additional negative going potentials of fixed latencies are also revealed. Responses to a second stimulus show a decrease or absence of N1-3 and no positive going potential. Application of CGP causes no apparent change in the response, but responses after a second stimulus show a small enhancement of N2+3. Interestingly, application of CGP in the presence of bicuculline results in a range of responses depending on the laminar placement of the recording electrode. Our results indicate that inhibitory synapses mediated by both $GABA_A$ and $GABA_B$ receptors play important roles in the processing of gustatory information in the vagal lobes of goldfish.

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293 Poster

ULTRASTRUCTURAL LOCALIZATION OF $GABA_B$ RECEPTORS IN THE RNST OF THE ADULT RAT.

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Previous light microscopic studies in our laboratory have demonstrated that $GABA_B$ receptor immunoreactivity in the adult rostral nucleus of the solitary tract (rNST) is present in neurons of all sizes as well as in their processes and has an interesting pattern of clustering. This study used electron microscopy and post-embedment immunogold labeling to determine the ultrastructural localization of $GABA_B$ receptors in the adult rNST. We successfully demonstrated $GABA_B$ receptors at the EM level; labeling was higher at synapses than in non-synaptic structures. The labeling is located at pre-synaptic terminals and/or post-synaptic profiles, as well as in the synaptic cleft. Labeled terminals are associated with dendrites, dendritic spines, and cell somata. Labeled terminals contacting other terminals are sometimes seen. This pattern likely represents pre-synaptic inhibition, a phenomenon believed to be mediated by $GABA_B$ receptors. We had previously identified two types of GABAergic terminals in the rNST as defined by their vesicular density: GABA-LD (low density) and GABA-HD (high density). They have a differential distribution such that GABA-LD terminals contact larger, more proximal dendrites and cell somata, while GABA-HD terminals contact smaller, more distal dendrites. We further suggested that GABA-LD terminals are dendrodendritic, while GABA-HD terminals are axodendritic. In the present study, we have determined that when $GABA_B$ receptors are both pre- and post-synaptic, the typical apposing dendrite has the cross-sectional dimensions of proximal dendrites and thus is likely GABA-LD. This is not true when $GABA_B$ receptor labeling is only pre- or post-synaptic. These data suggests that the pattern of labeling for GABA B receptors may be different for the two previously-defined GABAergic terminal types. It lends support to the hypothesis that GABAergic inputs to the proximal and distal dendrites may be different and reflect two GABAergic systems within the rNST capable of influencing individual neurons.

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294 Poster

DISTRIBUTION OF NK1 (SUBSTANCE P RECEPTOR) AND NK3 (NEUROKININ B RECEPTOR) IMMUNOREACTIVITY IN THE GUSTATORY ZONE OF THE NUCLEUS OF THE SOLITARY TRACT IN RATS.

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The tachykinins substance P (SP) and neurokinin B (NKB) are localized in fibers and terminals within the gustatory zone of the nucleus of the solitary tract (rNST) (Davis & Kream, 1993; Duncan et al, 1991; Lucas et al, 1992). SP is known to modulate gustatory neuron firing patterns (Davis & Smith, 1997; King et al, 1993), while the role of NKB in rNST is unknown. SP and NKB are primary ligands for the neurokinin receptors NK_1 and NK_3, respectively (Maggi & Schwartz, 1997). To identify potential sites of action of SP vs NKB in the rNST, we examined the distribution of their receptors by immunohistochemistry. Sections (50μ m) of rat brainstems were processed by standard methods for visualization of anti-NK_1 or anti-NK_3 antibodies (Novus Biologicals) with HRP, using DAB as substrate. Light microscopic examination revealed distinct but overlapping distributions of NK_3-immunoreactive (-ir) and NK_1-ir fibers and neuropil throughout the NST, both varying with rostro-caudal level over the extent of the nucleus. Near the area postrema in NST, NK_3-ir is widely distributed, in all subnuclei medial to the solitary tract, while NK_1-ir is more restricted. Conversely, in the caudal rNST, NK_3-ir is relatively restricted, concentrated dorsally in the central and medial rNST, while NK_1-ir is more extensive, especially ventrally. Both are sparse in lateral regions at this level. Rostral to IX^{th} nerve entry, the distributions appear co-extensive in the dorsal central region, but only NK_1-ir extends into lateral zones, while NK_3-ir is more abundant medially. Somata showing punctate NK_1 and NK_3 staining were seen, but regionally heavy staining of the neuropil precluded accurate determination of neuronal distributions. These data indicate that NK_3 as well as NK_1 receptors are localized within gustatory afferent termination zones in the rNST of rats, and suggest that NKB may have a significant role in gustatory processing.

295 Poster

TETANIC ELECTRICAL STIMULATION OF THE CHORDA TYMPANI ALTERS SINGLE UNIT RESPONSE PROFILES IN THE NUCLEUS OF THE SOLITARY TRACT OF THE RAT.

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Previous studies have shown that response profiles recorded from single taste-driven units in the nucleus of the solitary tract (NTS) exhibit gustatory context dependency. In other words, response profiles may change depending on the gustatory context in which stimuli are presented (e.g., following adaptation of the tongue to various taste stimuli). As NTS synaptic potentials have been observed to be complex mixtures of excitation and inhibition (Bradley and Grabauskas, 1998), NTS response profiles may be modulated by central inhibitory processes. The present experiment was designed to explore this hypothesis. Given that tetanic electrical stimulation of the rostral NTS has been shown to potentiate inhibitory activity within this nucleus in vitro (Grabauskas and Bradley, 1998), we developed an in vivo preparation which allowed us to deliver electrical pulse trains directly to single NTS units, but preserved the capability of recording taste responses from them as well. Urethane-anesthetized rats were prepared for electrical stimulation of the chorda tympani (CT) nerve by implanting electrodes into the inner ear. Single taste-driven NTS units were then isolated. Initially, electrophysiological responses were recorded to sucrose, NaCl, quinine, and HCl, presented in individual trials. Each trial consisted of a taste stimulus presentation followed by a distilled-water rinse. Next, each taste stimulus trial was preceded by brief (100-2000 msec) high-frequency tetanic (20-50 Hz) electrical stimulation of the CT. Finally, the response evoked by each taste stimulus was measured again. Preliminary data indicate that tetanic electrical stimulation had different effects on broadly and narrowly tuned units. For broadly tuned units, tetanus induced selective response suppression. For narrowly tuned units, tetanus frequently enhanced the response to the most effective stimulus and suppressed responses to other stimuli, rendering the unit even more narrowly tuned. These data are consistent with the hypothesis that response profiles in the NTS are modulated by inhibitory influences.

296 Poster

SODIUM GLUCONATE STIMULATES AMILORIDE-INSENSITIVE NEURONS IN THE RAT SOLITARY NUCLEUS

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At least two transduction pathways are hypothesized for sodium salts (e.g., Ye et al., *J. Neurophysiol.* 70:167-178, 1993). One well-defined transduction pathway involves the diffusion of cations through apically-located sodium channels on taste receptor cells. This mechanism is anion-independent and is blocked by the diuretic amiloride. A second mechanism is amiloride insensitive (AI) and may involve electroneutral diffusion of ions through the tight junctions between receptor cells. Supporting this hypothesis is the fact that sodium gluconate (NaG) evokes a minimal response in the whole chorda tympani (CT) nerve when amiloride is present. In hamster CT fibers, Na-acetate drives amiloride-sensitive (AS) N-fibers similarly to NaCl, whereas it is much less effective for AI H-fibers (Rehnberg et al., *J. Gen. Physiol.* 101:453-465, 1993). Cells in the nucleus of the solitary tract (NST) of the rat can be identified as either AS or AI, but are more broadly tuned than CT fibers. We recorded responses of single neurons in the NST and compared concentration-response functions for NaCl, NaG, and, when possible, KCl (0.01 - 1 M). Cells were classified as AS or AI, based on the effect of 30 μ M amiloride on responses to 0.1 M NaCl. Both the anterior tongue and nasoincisor ducts were stimulated with taste solutions for 10s. NaG elicited substantial responses in AI neurons. The ongoing response to NaG, like NaCl, was not blocked by amiloride in AI neurons. However, in AS cells, the responses to both NaG and NaCl were blocked by amiloride and on average these cells were more responsive to NaCl than NaG. These differences between the responses of NST neurons and those of the CT nerve could reflect functional differences between taste cells in the fungiform papillae and nasoincisor ducts, which is the subject of further investigation.

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297 Poster

DISTRIBUTION OF EFFERENT NEURONS PROJECTING TO AMYGDALA FROM BRAINSTEM NUCLEI INVOLVED IN CONDITIONED TASTE AVERSION EXPRESSION.

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The nucleus of the solitary tract (NTS), parabrachial nucleus (PBN) and amygdala play major roles in conditioned taste aversion (CTA) learning. To further understand these roles, we have begun to examine efferent projections to the amygdala from neurons within NTS and PBN that display fos-like immunoreactivity (FLI) following exposure to the unconditioned stimulus (US) or the conditioned stimulus (CS) after conditioning. Exposure to either the CS, saccharin, or the US, LiCl, elicited FLI primarily within the NTS parvicellular subnucleus (pc). Following injections of fluorogold (FG) into amygdala, a few cells per section were labeled within pc. Of these FG labeled cells, only 1-2 expressed FLI following administration of the CS, whereas almost all of the FG cells expressed FLI following US exposure. This finding indicates that some information concerning the US reaches the amygdala directly from NTS. It also suggests that cells in NTS which display FLI to the CS during CTA expression likely represent a different cell population and projection pattern than those which display FLI after LiCl. Within the PBN, exposure to the US elicited robust FLI within the external lateral-outer and some FLI within the external lateral-inner and external medial subnuclei. Following FG injections into amygdala and US exposure, most neurons were double-labeled within external lateral-outer, but few were double-labeled within the other two subnuclie. This result indicates that a subpopulation of neurons expressing FLI to the US in PBN project directly to amygdala. Thus, these experiments suggest that the gastrointestinal malaise signals elicited by the US reach the amygdala from the NTS via direct and indirect pathways. Furthermore, this study supports previous studies in suggesting that the amygdala plays a significant role in processing US signals during the acquisition of CTAs. Supported by NS37040 to ILB and DC02891 to CBH.

298 Poster

MODULATION OF PONTINE GUSTATORY NEURONS BY ELECTRICAL STIMULATION OF THE AMYGDALA IN RATS.

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The extracellular responses of single parabrachial neurons (PBN) were recorded during lingual application of sucrose, NaCl, NaCl mixed with amiloride, citric acid, and QHCl with or without concurrent electrical stimulation in the central nucleus of the amygdala (CeA). Based on the response characteristics of 51 PBN neurons, 3 neurons were classified as sucrose-best, 32 as NaCl-best, and 16 as citric acid-best. In most of the neurons sampled (90%), response rates to an effective stimulus were either inhibited or unchanged during stimulation pulses in the CeA. Specifically, a reduction in gustatory responsiveness was evident in 1 of 3 sucrose-best neurons, 18 of 32 NaCl-best neurons, and 15 of 16 citric acid-best neurons. The inhibitory modulation of NaCl-best neurons reduced the effectiveness of non-sodium stimuli relative to NaCl, thereby increasing the chemical selectivity of this physiological type. In 9 of the citric acid-best neurons, the inhibitory effect was more general, producing little change in the relative effectiveness of the stimuli tested. The other 6 neurons, however, changed from citric acid-best to NaCl-best during CeA stimulation pulses. Four of these returned to citric acid-best between pulses. In a smaller subset of NaCl-best neurons (n = 5), CeA stimulation augmented the responsiveness to NaCl. Taken together, stimulation of the CeA rendered NaCl a more salient stimulus in many NaCl-best neurons, but not in citric acid-best neurons. These findings provide an additional link between the amygdala and the maintenance of sodium balance; where activation of the CeA modulates a group of gustatory neurons known to play a role in NaCl discrimination and ingestion.

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299 Poster

THE MAJORITY OF TASTE-RESPONSIVE CELLS IN THE NUCLEUS OF THE SOLITARY TRACT OF THE HAMSTER PROJECT TO THE PARABRACHIAL NUCLEI

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From the nucleus of the solitary tract (NST) in the medulla, gustatory cells project to the parabrachial nuclei (PbN). Previous studies have shown in the rat that only a small proportion (25 - 45%) of taste-responsive NST cells are antidromically activated from the PbN. To more accurately stimulate the PbN, we positioned a bipolar stimulating electrode in the taste-responsive region of the hamster PbN under electrophysiological guidance. When a taste-responsive NST cell was isolated, current was applied to the PbN electrode to antidromically activate the NST neuron. The criteria for antidromic invasion were a constant latency and the ability to follow stimulus pulses at >100 Hz. For spontaneously firing cells, a collision test was conducted between spontaneous and stimulus-evoked action potentials. Taste solutions were 0.032M sucrose, NaCl, and quinine-HCl (QHCl), and 0.0032 M citric acid. Of 101 taste-responsive NST cells, 81 (80.2%) were antidromically activated. The mean (± SD) latency and antidromic threshold were 4.1 ± 3.7 ms and 48.3 ± 49.9 μ A, respectively. The mean conduction velocity of these cells was 0.76 ± 0.41 m/s. However, conduction velocities were bimodally distributed; QHCl-best cells showed significantly slower conduction than other cell types (t= 10.87, df= 79, p < .0001). The 23 QHCl-best neurons had a mean conduction velocity of 0.25±0.10 m/s, suggesting that they are considerably smaller than cells responding best to sucrose, NaCl, or citric acid, which were almost four times faster (0.95 ± 0.30 m/s). These results suggest that a large majority of taste-responsive NST cells are antidromically activated by PbN stimulation in the hamster when the site of PbN stimulation is determined by its gustatory responsiveness. Further, the slower conduction velocity of cells responding best to QHCl suggests that they may be smaller cells.

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300 Poster

CHRONIC MULTIELECTRODE RECORDINGS OBTAINED FROM THE GUSTATORY CORTEX OF BEHAVING RATS

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Current understanding of coding in the gustatory insular cortex (IC) primarily has come from acute single unit recordings. To extend our understanding of gustatory coding, we implanted bundles of microwires in rat dysgranular IC, and chronically recorded ensemble neural activity during self-administration of NaCl, sucrose, citric acid (CA), quinine HCl, and nicotine. Use of this approach allowed us to average responses over multiple trials of each stimulus, and to cross-correlate the responses of simultaneously recorded neurons. In agreement with previous single unit studies, we found that neurons showing large, long-lasting firing rate modulations to specific tastants comprised 8% of the sample. Averaging of multiple trials, however, permitted the quantification of more phasic firing rate modulations, including both excitation and inhibition. Such modulations increased the percentage of gustatory neurons to 28%.

Some neurons were excited by one tastant and inhibited by another, and some demonstrated differently timed responses to different tastants. For example, one neuron was inhibited for one time period following administration of 0.1 M NaCl and for another time period following administration of 0.2 M sucrose. Furthermore, tastant-specific firing coherence was observed between IC neurons. Some simultaneously recorded neurons fired synchronously to one stimulus (sucrose) but asynchronously to another (NaCl). Finally, a small subset of neurons (found in 3 animals) that did not respond to the tested stimuli did fire coherently with other neurons in a tastant-specific fashion. This finding suggests that gustatory coding may involve neurons that traditionally would not be characterized as gustatory.

The features of gustatory responses uncovered using chronic ensemble recording in behaving rats suggest that IC coding of tastants may involve not only long-lasting firing rate modulations, but also a variety of phasic changes and inter-neuronal interactions among a more distributed network of neurons.

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301 Poster

TASTE-RELATED FUNCTIONAL CHANGES IN THE HYPOTHALAMUS DURING INTAKE OF GLUCOSE, MONOSODIUM L-GLUTAMATE (MSG) AND NACL IN AWAKE RATS FED NORMAL AND NON-PROTEIN DIET.

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Umami (MSG) taste perception in the brain signals dietary protein intake. We developed functional magnetic resonance imaging technique (fMRI, 4.7 T) to determine oxygenation and blood flow changes in awake rats. Overnight fasted rats were fixed by the head platform attached with four pencil-like bars in the middle of the fMRI magnet. Rats ingested preferable taste solutions (0.06M MSG and NaCl, 0.6M glucose) or distilled water. Brain blood flow decreased in the hypothalamus in all groups following solution intake. As 8% neurons in the lateral hypothalamus (LH) differentially respond to umami taste stimulation, these findings suggest that umami taste perception could be integrated into the LH to recognize protein intake, same as saltiness serves to recognize electrolytes and sweetness energy sources. In addition, effects of taste solution intake on interstitial levels of norepinephrine (NE) were measured in the LH. Rats, housed in standard operant boxes, were fed either normal or non-protein diet for three days. Animals were without fluid access, besides a daily bar-mediated drinking session (75 min). Microdialysates, collected from the LH during the 75 min of drinking, were analyzed using HPLC. No significant responses of the LH NE to the drinking of distilled water, MSG, NaCl (0.06 M) and glucose solution were found in normally fed rats. However, a specific decline in LH NE release was detected during MSG solution-drinking in rats fed non-protein diet. Thus, LH NE may serve as a neuro-chemical substrate for association between umami preference and protein intake.

302 Poster

RESPONSES OF THE RAT CHORDA TYMPANI TO MIXTURES OF MSG AND SUCROSE IN THE PRESENCE OF AMILORIDE

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Monosodium glutamate (MSG) has a distinct taste that differs from the taste of sucrose and NaCl in humans (Hettinger et al., 1996). However, when mixed with 10-30μ M amiloride, an epithelial sodium channel blocker, the tastes of MSG and sucrose cross-generalize in laboratory rats (Yamamoto et al., 1991; Chaudhari et al., 1996). Rat chorda tympani (CT) responses to sodium salts, including MSG, are substantially reduced by amiloride. We recorded whole-nerve responses from 8 rat CT nerves to a concentration series (30-300mM) of sucrose, MSG, and sucrose-MSG binary mixtures. Each concentration of MSG was mixed with 300mM sucrose so that component concentrations in the mixture equaled stimulus concentrations presented alone. All solutions were tested in the presence and absence of 30m M amiloride. Each stimulus concentration series was bracketed by responses to 500mM NH_4Cl (standard response). Normalized CT responses to MSG were reduced 85% by amiloride; sucrose responses were not significantly affected. Responses to MSG-sucrose mixtures after amiloride were generally additive and larger ($p < 0.01$) than responses predicted by a single receptor model (Hyman and Frank, 1980). The overall average response to the 3 mixtures of 300mM sucrose with 30mM, 100mM, and 300mM MSG was 20.24% of the standard response, this is compared to 18.44% for the sum of responses to the components and 16.25% for a single-receptor model. Additivity held for sucrose mixtures containing 100 and 300mM MSG; however, 30mM MSG, which itself did not elicit a CT response after amiloride, enhanced ($p < 0.01$) responses to 300mM sucrose by 20%. Thus, transduction mechanisms for MSG and sucrose may interact. Furthermore, because of response additivity between sucrose and MSG we conclude that MSG may activate a separate receptor, such as the metabotropic glutamate receptor found in rat taste buds (Chaudhari et al., 2000).

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303 Poster

EFFECTS OF INJURY ON CHORDA TYMPANI SODIUM SALT RESPONSES

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We are interested in processes affecting peripheral nerve function as influenced by diet and damage to nerves and taste receptors in the oropharyngeal cavity. Previously, we showed that chorda tympani nerve responses to sodium salts are reduced when contralateral chorda tympani nerve section is combined with a low sodium diet (0.03%). In the current study, we have defined limits for this effect. Animals received unilateral nerve section of either the greater superficial petrosal or trigeminal nerve or a small burn (4 mm caudal from tip; 6 mm2) to the ventral tongue surface and placed on a low sodium diet. Whole nerve recordings from the left chorda tympani nerve were obtained 4-15 days after surgery. Chorda tympani responses to sodium stimuli in sodium restricted rats receiving unilateral trigeminal section or burn were specifically reduced to ~50% of their normal response levels; responses to non-sodium salts were similar to controls. Responses to all stimuli from greater superficial petrosal nerve section or sham-operated controls did not differ from those of normal adult rats.

The current study extended previous findings by demonstrating that damage to nerves in the tongue produce reduced neural responses that are specific to sodium salts, whether gustatory (chorda tympani, glossopharyngeal) or sensory (trigeminal). Interestingly, superficial damage to the ventral tongue surface also produced responses similar to nerve section, lending further support that there are non-specific, immune-related mechanisms responsible for the altered salt responses. In contrast, greater superficial petrosal nerve section produced no

deficit in chorda tympani nerve function illustrating that the palate does not contribute to the effect.

304 Poster

ETHANOL SUPPRESSES RESPONSES IN CATTLE AND MONKEY TASTE FIBERS TO COMPOUNDS BITTER OR SOUR TO HUMANS

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Previously we demonstrated that ethanol stimulated chorda tympani and glossopharyngeal taste fibers in several mammalian species. The response depended on the type of taste fiber. In monkeys ethanol suppressed responses to quinine hydrochloride (QHCl) in Q fibers and citric acid in H fibers (Hellekant et al. Alcohol, 1997, 14:473-484; Danilova and Hellekant. Alcohol, 2000, in press).

The purpose of this study was to investigate if ethanol suppresses only the responses to QHCl and citric acid or affects other compounds that taste bitter or sour to humans. Another purpose was to elucidate if this is a general mechanism, unrelated to species.

Method: Recordings were obtained from single chorda tympani fibers during stimulation with mixtures of a compound and 1 or 3 M ethanol. In monkeys we studied the effects with 2 acids: citric and ascorbic, and 7 bitter compounds: QHCl, denatonium benzoate, SOA, brucine, caffeine, aristolochic acid and naringin. In cattle we used 2 additional acids: butyric and propionic, and 3 bitter compounds: QHCl, urea, denatonium benzoate.

Results. The effects were similar in both species. In H fibers, addition of 1 or 3 M ethanol significantly inhibited responses to acids. In Q fibers responses to QHCl, denatonium benzoate, urea, brucine, aristolochic acid and naringin were suppressed by addition of ethanol. But the responses to caffeine and SOA were not affected. In fact, mixtures of ethanol and caffeine elicited even larger responses than caffeine itself. No suppression was observed in S fibers.

Conclusion. The results suggest that observed effects are unrelated to species. Further the effect of ethanol is not limited to one particular compound. It seems to be related to the taste quality of compound and therefore might reflect interaction of ethanol with specific taste receptors.

305 Poster

C57BL/6BYJ (B6) AND 129/J (129) MICE DIFFER IN THEIR WHOLE-NERVE CHORDA TYMPANI RESPONDING TO NATURAL AND ARTIFICIAL SWEETENERS

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Variation in preferences for sugars and other sweeteners is mediated in part by genetic factors in a number of species. In mice, the C57BL/6ByJ (B6) strain exhibits higher preferences for a variety of sweeteners than does the 129/J (129) strain. Gustatory neural responses to sucrose are also larger in B6 mice. We conducted an experiment to further examine differences between these two strains in neural responding to chemically disparate substances which humans would describe as sweet. Whole-nerve chorda tympani responses were significantly larger in the B6 group to lingual application of the sugars sucrose and maltose, the polyol D-sorbitol, and the noncaloric sweeteners acesulfame-K, SC-45647, and sucralose. However, responses tended to be similar between strains for a number of amino acids which are thought to taste sweet to mice, with the exception of L-proline, which elicited larger responses in the B6 group. Evoked activity was also larger in the B6 group to Polycose at 10%, but no differences were seen at 1% or 30%. The strains did not differ in their responding to NaCl, HCl, and quinine. Thus, there is variation in neural responding to sweeteners between the B6 and 129 strains which arises peripherally in the gustatory system and which in turn may underlie differences in their consumption of sweet-tasting substances, with the possible exception of amino acids.

306 Poster

SUCROSE OCTAACETATE (SOA) SENSITIVITY LOCUS (*SOA*) DETERMINES CHORDA TYMPANI AND GLOSSOPHARYNGEAL NERVE RESPONSES TO SOA IN SW.B6-*SOA*^{B} CONGENIC MICE: A GENETIC AND ELECTROPHYSIOLOGICAL STUDY

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In two-bottle preference tests, mice from the SWR/J (SWR) inbred strain avoid 0.01 - 1 mM SOA solutions, whereas mice from the C57BL/6J (B6) strain are indifferent to them. This difference is determined by allelic variation of a single gene, *Soa*, mapped to distal chromosome 6 (Azen, 1991; Capeless et al., 1992; Lush et al., 1995). The *Soa*^{a} (SWR) allele determines SOA avoidance, and the *Soa*^{b} (B6) allele determines indifference to SOA. The SW.B6-*Soa*^{b} congenic mouse strain has been selected using a backcross-intercross system to transfer a *Soa*-containing donor chromosome fragment from the B6 strain on the genetic background of the SWR strain (Harder et al., 1996). As a result, the SW.B6-*Soa*^{b} mice do not avoid SOA in the two-bottle tests, unlike the mice from the inbred partner SWR strain. Using PCR-based microsatellite DNA markers polymorphic between the parental B6 and SWR strains, we determined the length and location of the B6 donor chromosome fragment. It is flanked by *D6Mit109* (61.4 cM from centromere) proximally and *D6Mit57* (71.1 cM from centromere) distally and thus spans less than 9.7 cM of distal chromosome 6. Electrophysiologically-recorded responses of the whole chorda tympani and glossopharyngeal nerves to lingual application of 1 mM SOA were smaller in SW.B6-*Soa*^{b} congenic mice compared with the SWR mice. This suggests that the effect of the *Soa* locus on SOA avoidance is mediated by peripheral taste responsiveness. Most likely, the *Soa* locus affects taste receptor cell populations innervated by both chorda tympani and glossopharyngeal nerves.

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307 Poster

EFFECTS OF BRIEF PULSES OF TASTE STIMULI ON SUBSEQUENT TASTE RESPONSES IN THE CHORDA TYMPANI OF THE RAT

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Recent data from our lab has shown that the magnitude of taste responses in the nucleus of the solitary tract (NTS) depends on the identity of the previously presented stimulus. That is, data suggests that gustatory neural activity in the NTS can change depending on the context in which a taste stimulus is presented. This property may be the result of complex interactions of excitation and inhibition within the NTS or, alternatively, may reflect interactions at the receptors or among peripheral nerve fibers. In order to determine the contribution of peripheral interactions to the context-dependency observed in the NTS, neural activity was recorded from the whole chorda tympani (CT) nerve in anesthetized rats. Taste stimuli consisted of sapid solutions of NaCl (.1 M), HCl (.01 M), sucrose (.5 M), quinine HCl (.01 M) and NH4Cl (0.5 M). Initially, each tastant was presented individually for 10 sec. Next, each tastant (test stimulus) was presented for 8 sec following ,after either 1 or 5 sec, a 100 msec presentation (prepulse) of either NaCl, HCl, quinine, sucrose or dH2O. A dH2O rinse was presented in the interval between the prepulse and the test stimulus. Responses to NH4Cl were periodically recorded to ensure the recording stability. Results show that brief pulses of sucrose can suppress subsequent responses to quinine in the CT. This effect was also noted in the NTS. However, in the CT but not the NTS, brief pulses of quinine can suppress subsequent responses to sucrose as well. Sucrose prepulses did not attenuate the response to sucrose nor did quinine prepulses attenuate the response to quinine. Other stimulus-stimulus interactions were apparent. These data suggest that the effects of taste stimulus prepulses on taste responses in the NTS may be in part the result of interactions that occur at the periphery.

308 Poster

BIOPHYSICAL PROPERTIES AND RESPONSES TO GLUTAMATE RECEPTOR AGONISTS OF IDENTIFIED SUBPOPULATIONS OF RAT GENICULATE GANGLION NEURONS

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The goal of the current study was to evaluate the electrophyisological properties and responses to glutamate receptor agonists of neurons of the rat geniculate ganglion (GG) innervating the tongue. This was accomplished with whole-cell recordings on acutely dissociated neurons. Subpopulations of GG neurons were labeled by injecting Fluoro-Gold (FG) or True Blue chloride into the anterior tongue (AT neurons) and soft palate (SP neurons) and applying FG crystals to the cut end of the posterior auricular branch of the facial nerve (PA neurons). GG neurons had a resting membrane potential of -55.3 ± 0.7 mV (SE), an input resistance of 339 ± 12 MV and an action potential amplitude of 63.6 ± 0.9 mV. Although many biophysical properties of the AT, SP and PA neurons were similar, significant differences were found among these groups related to cell excitability. For example, the average amount of current necessary to elicit an action potential was 61 pA in AT neurons (n = 55), 90 pA in SP neurons (n = 41) and 189 pA in PA neurons (n = 35, $p < 0.001$). In addition, AT neurons tended to fire significantly more action potentials during depolarization as well as following hyperpolarizing pulses than SP or PA neuron types. These results suggest that subpopulations of neurons in the geniculate ganglion have distinct biophysical properties possibly related to their functional heterogeneity.

Most GG neurons responded to application of glutamate receptor agonists. The neurons responded with a depolarization accompanied by a reduction in input resistance. The responses of the AT, SP and PA neuron subpopulations were similar. These results indicate that cell bodies of GG neurons express functional glutamate receptors. Therefore, glutaminergic neurotransmission may play a role in the processing of gustatory, and other sensory information, within the geniculate ganglion and its projections.

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309 Poster

REAL-TIME MEASUREMENT OF NEUROTRANSMITTER RELEASE FROM RAT TASTE BUDS

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Receptor cells in taste buds (TBs) form connections with sensory afferent fibers. Taste cells may also synapse with other cells within the taste bud or with efferent fibers. The identity of neurotransmitters at these synapses is not yet known although a number of transmitter candidates have been suggested. To date, the most compelling experimental evidence exists for serotonin, based mainly on immunocytochemical and radioligand-uptake/release studies. Functional experiments directly measuring transmitter release will aid in unambiguous identification of neurotransmitters released at TB synapses. We have employed cyclic voltammetric techniques using fine-tipped (5 μ m) carbon-fiber electrodes (CFEs) to study the release of aminergic neurotransmitters from TBs in real time. Previously, we reported stimulus-dependent responses from TBs indicating release of one or more unidentified oxidizable substrates, possibly including biogenic amines (Jafri & Roper, 1999). The responses were calcium dependent and TB specific. To establish the identity of synaptically released transmitters, we have now extended these findings by manipulating amine synthesis and uptake pathways using selective pharmacological tools. Using Nafion-coated CFEs, we recorded responses elicited by depolarizing TBs with 50-100 mM KCl. Adding clomipramine (2 nM), a selective serotonin uptake inhibitor, to the bath during recording enhanced responses to KCl stimulation. In contrast, adding imipramine (2 nM), a selective norepinephrine uptake inhibitor, did not alter responses. Additionally, isolated TBs were preloaded with serotonin by incubating them with serotonin (500 μ M) or 5-hydroxy-L-tryptophan (2 mM), a serotonin precursor, for 30 minutes before recording. These treatments also enhanced responses elicited by depolarizing TBs. When TBs were incubated with serotonin in the presence of clomipramine, we did not observe enhanced responses to depolarization, providing further evidence that serotonin-specific mechanisms are present in TBs. In conclusion, these data provide strong evidence that serotonin is among the neurotransmitters released from TBs.

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310 Poster

CALCIUM IMAGING REVEALS SYNAPTIC GLUTAMATE RECEPTORS IN TASTE CELLS

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The neurotransmitter(s) implicated in synaptic transmission in taste buds are not known. Recently, we showed that a subset of taste cells express Ca^{2+}-permeable glutamate receptors (GluRs) of the non-NMDA type (Caicedo et al., J Comp Neurol, 2000). In the present study, we have developed a new calcium imaging approach that allows us to test if functional GluRs are present in taste cells. Taste cells of foliate papillae were loaded with calcium green dextran (CaGD, 5 mM). Slices of foliate papillae containing CaGD-loaded taste cells were imaged with a scanning confocal microscope. We tested if depolarization reliably increased $[Ca^{2+}]i$ in CaGD-loaded cells by superfusing the preparation with Tyrode[tm] solution containing 50 mM K+. Depolarization of taste cells increased CaGD fluorescence from baseline by 26.4% ± 4.1 (mean ± s.e.m.). Elevating Ca^{2+} in the bath to 8 mM markedly increased depolarization-induced CaGD fluorescence changes to 83.8% ± 11.4. Responses were reversibly abolished when Ca^{2+} was omitted from the bathing solution. To test whether activation of GluRs induced changes in $[Ca^{2+}]i$ we perfused slices with Tyrode[tm] solution containing glutamate (30 mM - 1 mM) or the non-NMDA receptor-specific agonists kainate (KA, 30-100 μ M) or AMPA (30-100 μ M). Responses were observed in 31% of the cells (11/35) tested with 300 mM glutamate. The average increase in $[Ca^{2+}]i$ was 8.7% ± 1.2 in response to 300 mM glutamate. Responses could be induced by glutamate concentrations as low as 30 mM. Glutamate induced $[Ca^{2+}]i$ changes in a concentration-dependent manner. The non-NMDA receptor antagonists CNQX (10 μ M, n = 3) and GYKI 52466 (10 μ M, n = 3) reversibly blocked responses to glutamate. Finally, stimulating with KA (100 mM) increased $[Ca^{2+}]i$ by 8.7% ± 3.4. These experiments indicate that there are GluRs, possibly AMPA and KA subtypes, in taste cells.

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311 Poster

A NEW APPROACH FOR IMAGING CA^{2+} IN TASTE CELLS REVEALS SYNAPTIC GLUTAMATE RECEPTORS

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The neurotransmitters at synapses in taste buds are not yet known with confidence. Recently, we showed that taste cells take up cobalt when stimulated with glutamate, presumably through synaptic glutamate receptors (GluRs, Caicedo *et al.*, J Comp Neurol, 2000). Here we report a new calcium imaging technique for taste buds that allows us to test the presence of these GluRs in living isolated tissue preparations. Taste cells of foliate papillae were loaded with calcium green dextran (CaGD). Lingual slices containing CaGD-labeled taste cells were imaged with a scanning confocal microscope. Superfusing the preparation with Tyrode's solution containing 50 mM K^+ (to depolarize taste cells) increased CaGD fluorescence by 26.4% ±4.1 (mean ±s.e.m.). Elevating Ca^{2+} in the bath to 8 mM markedly increased depolarization-induced CaGD fluorescence changes, and responses were reversibly abolished when Ca^{2+} was omitted from the bathing solution. These observations are consistent with responses to depolarizing K^+ solutions being generated by Ca^{2+} influx through voltage-gated Ca^{2+} channels. To activate GluRs, we perfused slices with glutamate (30 μ M to 1 mM), kainate (30,100 μ M), or AMPA (30,100 μ M). Responses were observed in 31% of the cells (11/35) tested with 300 μ M glutamate. Glutamate responses were dose-dependent and were induced by concentrations as low as 30 μ M. The non-NMDA receptor antagonists CNQX (10 μ M, n = 3) and GYKI 52466 (10 μ M, n = 3) reversibly blocked responses to glutamate. Finally, stimulating with kainate or AMPA also elicited Ca^{2+} responses. These results indicate that, consistent with our previous study using Co^{2+} uptake, there are GluRs, possibly AMPA and KA subtypes, in taste cells. Collectively, the data suggest that glutamate is a neurotransmitter in taste buds. The function of GluRs in taste buds is not yet known. GluRs might be presynaptic autoreceptors or postsynaptic receptors at intragemmal or efferent synapses.

312 Poster

IONIC DEPENDENCE OF THE PROTON-ACTIVATED CURRENT IN RAT VALLATE TASTE CELLS

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Recently two novel channels have been proposed to transduce sour taste in rodents: a mammalian degenerin-1 (MDEG1, also known as BNC1 or ASIC2a, Ugawa et al., Nature, 395:555-556, 1998), and an NPPB-sensitive Cl^- conductance (Okada et al., J Neurophysiol. 80:1852-1859, 1998). Previously, we showed that most rat taste cells of vallate papillae respond to a pH drop from 7.4 to 5 with an inward current accompanied by membrane depolarization. This current shares some physiological properties with recently cloned acid-sensing ion channels (ASICs) that are proton-gated Na^+ channels (Lin and Kinnamon, Chem. Senses, 24:570, 1999; Waldmann and Lazdunski, Curr. Opin. Neurobiol. 8:418-424, 1998). In this study we investigated the ion conductances involved in the proton-induced response in rat vallate taste cells. Under voltage-clamp conditions, proton-activated currents reverse at approximately 45 mV, a potential relatively close to the Na^+ equilibrium potential. To monitor intracellular Na^+ levels directly, we loaded taste cells with the Na^+-sensitive dye SBFI and examined proton-induced responses using Na^+ imaging. Citric acid (pH 5) increased the intracellular Na^+ level, and the increase was pH-dependent. These data indicate that Na^+ influx is involved in the proton-induced response. In addition, we investigated the possible contribution of Cl^- channels. The Cl^- channel blocker NPPB partially blocked the proton-induced current. However, the effect of NPPB persisted even when the cells were held at the Cl^- equilibrium potential (E_{Cl^-}), where there is no net Cl- current. Furthermore, changes in ECl- failed to shift the reversal potential of the proton-induced current and did not alter the amplitude of the response significantly. These data indicate that NPPB may block conductances other than Cl- channels. Taken together, proton-activated Na+ channels appear to play an important role in the sour response in vallate taste cells.

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313 Poster

ACETYLCHOLINE INCREASES INTRACELLULAR CALCIUM LEVELS VIA MUSCARINIC RECEPTORS IN TASTE RECEPTOR CELLS

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Neuroactive compounds have been found in taste buds and are proposed to modulate taste responses in taste receptor cells (cf. Nagai et al., Chem. Senses 21:353-365, 1996). Choline acetyltransferase, a key biosynthetic enzyme for acetylcholine (ACh), has been found in taste bud cells and in axons innervating taste buds in rats and mice (Kim and Roper, Soc. for Neurosci. Abstr. 20:981, 1994). However, only a few studies have examined the effects of ACh at the receptor cell level. In previous studies, the ACh receptor agonist carbachol activated PI turnover in rat taste buds (Hwang et al., Proc. Natl. Acad. Sci. USA 87:7395-7399, 1990), and ACh and a muscarinic ACh receptor agonist decreased Cl^- conductance and hyperpolarized mudpuppy taste cells (Ewald & Roper, Soc. Neurosci. Abstr. 20:980, 1994). In this study, I examined physiological responses to ACh in mudpuppy taste cells by measuring $[Ca^{2+}]_i$ with the Ca^{2+}-sensitive dye fura-2. ACh increased $[Ca^{2+}]_i$ levels. Atropine, a muscarinic ACh inhibitor, blocked the ACh response, but the nicotinic inhibitor d-tubocurarine had no effect. These data suggest that the response is mediated via a muscarinic ACh receptor. U73122, a phospholipase C inhibitor, blocked the ACh response. Also, thapsigargin, a Ca^{2+} ATPase inhibitor that depletes intracellular Ca^{2+} stores blocked the response to ACh. These results suggest that ACh binds to a muscarinic receptor, which is likely of the M1/M3/M5 receptor subtype. Receptor binding leads to activation of phospholipase C, thereby increasing IP_3 to release Ca^{2+} from intracellular stores. Previously, we demonstrated that bitter compounds increase $[Ca^{2+}]_i$ via the IP_3 pathway in mudpuppy taste cells (Ogura et al., J. Neurosci. 17:3580-3587, 1997). Therefore, effects of ACh on $[Ca^{2+}]i$ levels could modulate bitter taste transduction in mudpuppy taste receptor cells.

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314 Poster

CHOLECYSTOKININ INCREASES INTRACELLULAR CALCIUM LEVELS IN RAT POSTERIOR TASTE RECEPTOR CELLS.

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Our laboratory has previously localized the neuropeptide cholecystokinin (CCK) to taste receptor cells (TRCs) using both immunocytochemistry and RT-PCR. To investigate its physiology, calcium-imaging studies were performed using TRCs dissociated from posterior rat tongue. Standard ratiometric techniques using the fluroprobe fura-2 at 340/380 excitation wavelengths were employed. Sulfated CCK octapeptide was exogenously applied to TRCs using a pipette positioned close to the cell. Images were obtained once every ten seconds during the stimulation period. CCK was tested at three concentrations. At the highest concentration, 10^{-5}M, 11 of 49 tested taste receptor cells responded with increases of intracellular calcium. At 10^{-6} M, 13 of 78 cells responded to exogenously applied CCK. With eight cells a second application of CCK was possible; 7 responded a second time. Responses of 10 of 58 cells were recorded to 10^{-7} M CCK. Four of six tested cells responded to a second application. Thus about 18% of cells responded at all concentrations (34 of 185 tested cells). At all concentrations, responses were spike-like; although CCK presentation was maintained, responses peaked and returned to baseline within 58±3.5 seconds (n = 33). Latencies of response to CCK superfusion varied from 1 to 10 minutes (5.25±0.7 minutes; n = 34). Experiments are in progress to determine whether responses require extracellular or intracellular calcium. Nineteen taste receptor cells that responded to CCK were also tested to caffeine. Eleven of 19 responded to concentrations from 1, 5, or 10 mM. Additionally, three of seven cells that responded to CCK also produced responses to quinine (0.2 or 1 mM). These experiments show for the first time that posterior taste receptor cells respond to CCK, presumably using the IP_3 second messenger system, and that some of these cells also possess bitter sensitivity.

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315 Poster

LINGUAL SURFACE PH AFFECTS INTRACELLULAR PH (PH_i) IN POLARIZED TASTE RECEPTOR CELLS (TRCS)

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In isolated TRCs pH_i is affected by changing solution pH (Lyall et al., AJP 273: C1008-C1019, 1997). We now investigate whether lingual surface pH (pH_{ls}) affects pH_i in polarized TRCs. pH_i was monitored in polarized fungiform papillae loaded with the pH-sensitive fluoroprobe, BCECF. Polarity of the papillae was maintained by mounting a piece of isolated rat lingual epithelium containing a single papilla in a special microscopy chamber (Chu et al., AJP 269: C1557-C1564, 1995). Lingual and basolateral surfaces of the papilla were perfused independently with HCO_3—free HEPES buffered media (pH 7.4; 22°±1°C). The cells were imaged through a 40X objective from the basolateral side at 510 nm with an intensified CCD camera as they were excited alternately at 490 nm and 440 nm. pH_i was monitored with the fluorescence emission ratio (F_{490}/F_{440}).

Decreasing pH_i from 7.4 to 5.3 reduced TRC pH_i by 0.2 pH unit, while decreasing pH_{ls} from 7.4 to 3.0 reduced pH_i by 0.3 unit. The change in pH_i per unit pH_{ls} ($D\ pH_i/D\ pH_{ls}$) was 0.08. Decreasing pH on the basolateral surface (pH_{bs}) from 7.4 to 6.7 reduced pH_i by 0.56 pH unit, and $D\ pH_i/D\ pH_{bs}$ was 0.8. Therefore, in polarized TRCs changes in pH_i differ significantly depending on which external environment is altered, unlike isolated TRCs and symmetrical nontaste H^+-responsive chemosensory cells. Moreover, the solution pH on the lingual surface affects pH_i significantly less than the pH on the basolateral surface. This suggests that the apical membrane of TRCs is less permeable to H^+ ions than the basolateral membrane, protecting TRCs from injury at low lingual surface pH. This low apical membrane permeability to H^+ ions also accounts for the low surface pHs necessary to evoke robust sour taste responses.

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316 Poster

NA^+- H^+ EXCHANGE ACTIVITY IN THE BASOLATERAL MEMBRANE OF TASTE RECEPTOR CELLS (TRCS)

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To identify whether Na^+ - H^+ exchange activity is present in TRCs, intracellular pH (pH_i) was monitored in polarized fungiform papillae loaded with the pH-sensitive fluoroprobe, BCECF. The polarity of the papillae was maintained by mounting a piece of isolated rat lingual epithelium containing a single papilla in a special microscopy chamber (Chu et al., AJP 269: C1557-C1564, 1995). Apical and basolateral sides of the papilla were perfused independently with HCO_3—free HEPES buffered media (pH 7.4; 22°±1°). The cells were imaged from the basolateral side through a 40X objective at 510 nm with an intensified CCD camera as they were excited alternately at 490 nm and 440 nm. pH_i was monitored with the fluorescence emission ratio (F_{490}/F_{440}).

Several lines of evidence indicated that Na^+-H^+ exchange activity is present in the basolateral membrane of TRCs. 1) Removing Na^+ from the basolateral perfusate by substituting NaCl with equimolar NMDG-Cl decreased pH_i, and amiloride,a Na^+-H^+ exchanger inhibitor, attenuated that decrease in pH_i. 2) TRC pH_i also decreased when amiloride was added to Na^+ containing perfusate. 3) Acid loading of TRCs by pulsing with 15 mM NH_4Cl or by exposing to 15 mM sodium acetate induced transient decreases in TRC pH_i that recovered spontaneously to baseline values. The spontaneous recovery of TRC pH_i was blocked by the addition of amiloride to the basolateral perfusate, and was blocked by the removal of Na^+ from the basolateral perfusate. Removal of solution Cl^- had no effect on the response to acid loading or spontaneous pH recovery. Thus, at constant extracellular pH intracellular acid-base balance of TRCs is maintained by Na^+-H^+ exchange activity in the cell basolateral membranes.

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317 Poster

AQUAPORIN EXPRESSION AND HYPOOSMOTIC-INDUCED CURRENTS IN NON-LINGUAL TASTE BUDS

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Recently, we have demonstrated the presence of several subtypes of aquaporins (water channels) in lingual taste buds and shown effects of hypoosmotic stimuli at the cellular level (Gilbertson et al., *Chem. Senses* 24:569, 1999). However, it is generally believed that other areas in the oral cavity, particularly the epiglottis, larynx and nasopharnyx may be more important in mediating the response to water than those in the tongue. In order to determine if these areas also contain aquaporins and respond to hypoosmotic stimuli, we have a combination of immunocytochemistry and patch clamp recording on taste buds obtained from non-lingual areas. We have stained rat taste buds isolated from the soft palate and epiglottis using antibodies against aquaporins (AQP) -1, -2, and 5, which we previously found in lingual taste buds. Similar to the pattern of staining seen in posterior rat lingual taste buds, palatine taste buds, including those from the Geschmacksstreifen, showed AQP-1 and -2 labeling on the basolateral regions of the cells, while labeling with anti-AQP-5 antibodies was predominately apical. Epiglottal taste buds also labeled with all three AQP antibodies. Though there was a similar regional distribution of labeling in epiglottal taste buds, it was less clear than in the case of those from the palate due to their comparatively smaller size. Patch clamp recording was performed on taste buds isolated from the palate, epiglottis and nasopharnyx to determine if hypoosmotic stimuli had effects on taste cells from these areas. Currents elicited in response to voltage ramps (-90 to +60 mV) were recorded in control saline and in saline solutions varying only in osmolarity (-90 mOsm) from control. Similar to that reported in lingual taste buds, hypoosmotic stimuli activated a depolarizing conductance in roughly half of the cells from these three areas.

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318 Poster

HYPOOSMOTIC STIMULI ACTIVATE A CHLORIDE CURRENT IN TASTE CELLS

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Recently we proposed a model to account for water (hypoosmotic) responses in taste receptor cells involving water movement through aquaporin water channels, taste cell swelling and activation of a depolarizing conductance (Gilbertson *et al.*, Chem. Senses 24:569, 1999). In the present study, we have attempted to characterize the conductance activated in the presence of hypoosmotic stimuli in greater detail using whole-cell patch clamp recording on isolated rat taste buds. Currents elicited in response to voltage ramps (-90 to +60 mV) were recorded in control saline and in saline solutions varying only in osmolarity (-30, -60 and -90 mOsm) from control. In roughly half the cells, hypoosmotic solutions caused a 15% increase in cell surface area (e.g 'stretch') and activated a reversible conductance that exhibited marked adaptation in the continued presence of the stimulus. Ion substitutions experiments were consistent with the interpretation that the predominant ion carried through these apparent stretch-activated channels was Cl-. Reversal potentials for the hypoosmotic-induced current closely matched those predicted by the GHK constant field equation for a Cl- conductance. In addition to Cl-, SCN-, I- and Br- were also significantly permeant through these channels, while isethionate- was comparatively less permeant. Pharmacological experiments revealed that this Cl- conductance was inhibited by 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) and 5-nitro-3-(3-phenylpropylamino)benzoic acid (NPPB) (EC_{50} = 1.65 μ M and 5.07 m M, respectively), but not to $CdCl_2$ (300 m M) nor $GdCl_3$ (200 m M). We hypothesize that this stretch-activated Cl- conductance represents the transduction mechanism by which the presence of hypoosmotic stimuli, including water, may be signaled in taste receptor cells.

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319 Poster

THE ROLE OF ROD α-TRANSDUCIN IN TASTE SIGNAL TRANSDUCTION

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Rod α-transducin is a G-protein α -subunit well known for its role in phototransduction. α-Transducin is 80% identical to α -gustducin, a G-protein α subunit that plays a key role in transducing responses to bitter and sweet compounds. Several lines of evidence suggest that α-transducin may also play an important role in taste signal transduction. α-Transducin is expressed in rat taste receptor cells and studies to date suggest that α-gustducin and α-transducin are biochemically indistinguishable. In comparison to wildtype mice, behavioral and taste nerve responses of α-gustducin null mice to compounds that humans consider sweet or bitter were greatly reduced, but not totally abolished. At high concentrations of tastants, the α-gustducin knockout mice avoided bitter compounds and preferred sweet compounds indicating that molecules in addition to α-gustducin are involved in signal transduction of these bitter and sweet compounds. Furthermore, transgenically expressed dominant negative mutants of α-gustducin inhibited taste responses of wildtype and α-gustducin knockout mice, arguing that other G-proteins in addition to gustducin are involved. In α-gustducin null mice transgenically expressing α-transducin under the control of the α-gustducin promoter, the responses to sucrose, SC45647 and denatonium benzoate were partially restored, although the responses to quinine were not. These results suggest that there may be functional differences between α-transducin and α-gustducin.

To more directly evaluate the contribution of α-transducin to taste responses in vivo we generated α -transducin knockout mice in which all of Exons 4 and 5, and parts of Exons 3 and 6 (corresponding to α-transducin amino acids 64-206) were deleted and replaced with a PGK-Neo cassette. These α-transducin knockout mice have been crossed with α-gustducin knockout mice to produce α-transducin/α-gustducin double knockouts. Behavioral and nerve recording experiments with knockout mice lacking α-gustducin, or α-transducin, or both G protein α-subunits are in progress.

320 Poster

BITTER TASTE TRANSDUCTION USES TWO SECOND MESSENGER SYSTEMS

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It is now clear that bitter taste transduction uses multiple mechanisms. For this study, vallate and foliate taste and adjoining non-taste lingual tissues from SWR mice were used. Epithelium was peeled off the posterior tongue using collagenase. Taste and non-taste tissues were surgically isolated and homogenized. Second messengers were analyzed in real time (msec) using a the BioLogicQFM. The bitter stimulus, denatonium, induced a rapid (75-100msec) and transient rise of the second messenger, IP3, in taste tissue. Denatonium also induced a rapid (50msec) drop in cAMP levels, suggesting an involvement of α-gustducin in this step. Antibodies to α -gustducin, but not preimmune IgG, inhibited this drop in cAMP but were ineffective in altering the rise in IP3. It was previously demonstrated that the rise in IP3 is due to stimulation of a PLC by a β g -G protein subunit whose g -subunit is a taste specific g 13 (Huang et al., Nature Neurosci. 2:1055, 1999). Antibodies to this g 13 inhibited the denatonium-induced rise in IP3. The observation that a b g -subunit could activate a PLC and the finding that taste cells contain a PLCb 2 isozyme, prompted us to use antibodies specific to several members of the PLC family to identify isozyme(s) responsible for bitter-stimulated rise in IP3. Antibodies to the isoforms, PLCb 2, PLCb 3, and PLCb 4, and their respective blocking peptides, demonstrated that only the antibodies to PLCb 2 prevented the denatonium-induced rise in IP3. Collectively these observations suggest a mechanism for bitter taste transduction involving two messenger systems. Denatonium induces an a -gustducin mediated drop in cAMP along with a b g 13-subunit mediated, PLCb 2 enzymatic rise in IP3. Increased IP3 may release calcium from intracellular stores, while low cAMP may stimulate cyclic-nucleotide-suppressible ion channels, all of which lead to cellular depolarization and release of neurotransmitter.

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321 Poster

EXPRESSION OF GENES INTRODUCED INTO RAT TASTE CELLS VIA LIPOSOME- MEDIATED TRANSFECTION

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Recently, cDNAs for many proteins, potentially important in taste transduction, have been cloned from mammalian taste buds. Re-introducing and expressing such cDNAs in taste buds would allow one to evaluate the functional properties of the gene products in their native environment. Because there are no cultured lines of taste cells, we have developed a method of transfecting rat taste cells in primary culture. We have used liposomes to introduce plasmids and express either β-galactosidase (β-gal) or enhanced green flourescent protein (EGFP) as reporters of transfection. Transfections were successfully performed in a whole mount lingual epithelial preparation as well as in isolated taste buds. We find that transfection efficiency in both preparations increases if the delaminated epithelium is re-exposed to fresh enzyme (Collagenase, Dispase II, Trypsin Inhibitor) cocktail. This may result in the removal of extracellular matrix components, allowing liposomes direct access to the cell membrane. Optimal redigestion varies depending on the animal's age and on the source of taste buds (foliate, circumvallate, and fungiform) and is controlled by varying the time of digestion. Taste cells are transfected with liposomes during an overnight culture. Dramatically different efficiencies of expression of reporter genes were noted with several lipid reagents tested (Cellfectin, DMRIE-C, Lipofectin, Lipofectamine, and GeneShuttle). We have used immunocytochemistry for G α-gustducin to confirm that taste cells indeed are transfected. Taste cells are more efficiently transfected as isolated taste buds rather than in a whole mounted epithelium, presumably because of better access. This method should permit new strategies for examining the functions of cloned cDNAs.

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322 Poster

CAPSAICIN (VR1) RECEPTORS IN TASTE BUDS: FUNCTION AND LOCALIZATION

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When capsaicin, the active principal of hot peppers, is applied to the tongue, it alters the perception of classical taste substances. Whether this change is due to peripheral or central events is unclear. In order to study this phenomenon, we measured the changes in chorda tympani single unit responses to classical tastants applied before or after application of capsaicin. In addition, we utilized immunohistochemistry and RT/PCR to study the distribution of the capsaicin receptor, VR1.

Some single units of the chorda tympani nerve in rats change activity in response to lingual application of 10 μ M capsaicin. Other chorda tympani units that do not respond directly to capsaicin show decreased responsiveness to 0.1 M NaCl and other tastants following application of capsaicin. These results imply the presence of receptors on either taste receptor cells or gustatory nerve fibers, or some non-specific inhibitory effect. In order to determine the distribution of VR1 in rats, we carried out RT/PCR on trigeminal and geniculate ganglia, and on taste buds punched from fungiform papillae as well as on non-taste epithelium. These studies show that VR1 mRNA is detectable in both ganglia as well as in both taste and non-taste epithelia. In addition, immunocytochemistry was carried out using an antiserum to VR1 (from R. Elde). For these experiments, fungiform, foliate and vallate papillae were fixed in 4% buffered paraformaldehyde with and without 0.2% picric acid. Numerous immunoreactive nerve fibers were present both within and around the taste buds. Further, occasional elongate taste cells exhibited membrane-associated immunoreactivity along their entire height. Taken together, these results demonstrate that the VR1 capsaicin receptor is present on taste cells as well as the intragemmal and perigemmal nerve fibers. Thus perceptual interactions of capsaicin with classical tastants may involve a significant peripheral component.

323 Poster

APPLICATION OF SEMLIKI FOREST VIRUS SYSTEM FOR EXPRESSION OF ODORANT AND TASTE RECEPTORS

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The highly efficient Semliki Forest Virus (SFV) system has been applied for expression of many 7TM receptors. The broad host range of SFV has allowed studies of recombinant proteins in many cell lines and primary cultures. We used SFV system to express the rat I7 odorant receptor and assay its localisation and functional responses. Immunofluorescence and confocal microscopy revealed that, in contrast to most cell lines, when the embryonic olfactory epithelium (OE) cultures were used for expression of the I7GFP fusion protein, the receptor was localised at the plasma membrane. Rat OE, and the primary cultures derived from the mature OE neurons were successfully infected with SFV-LacZ virus. When the OE neurons were infected with SFV-I7 and SFV-I7GFP, functional responses to the I7 ligands octanal and nonanal were observed.

It has been long suggested that certain chemical stimuli are transduced via G-protein-coupled receptors of the taste cells. A truncated mGlu4-like receptor, cloned from the rat taste buds, is implicated in the monosodium glutamate taste responses. We expressed the related full-length rat brain mGlu4 in SFV system and investigated the effect of several peptides with alleged umami properties on the [^{3}H]-L-AP4 binding and receptor functional activity using the GTPγ ^{35}S assay. By comparing the binding results to the functional studies, we demonstrated the corresponding potencies for the agonists L-AP4 and L-glutamate. Several of the umami peptides were identified as mGlu4 agonists, albeit with varying potencies and more important very different efficacies. The independent taste evaluation of the same peptides demonstrated that, in agreement with its efficacy on the mGlu4 receptor, only Glu-lac tasted distinctly umami, similar to MSG. The combination of the taste assessment and in vitro receptor binding and activation results suggest that a mGlu4 receptor similar to the one in brain might be involved in transducing the umami taste stimulus.

INDEX