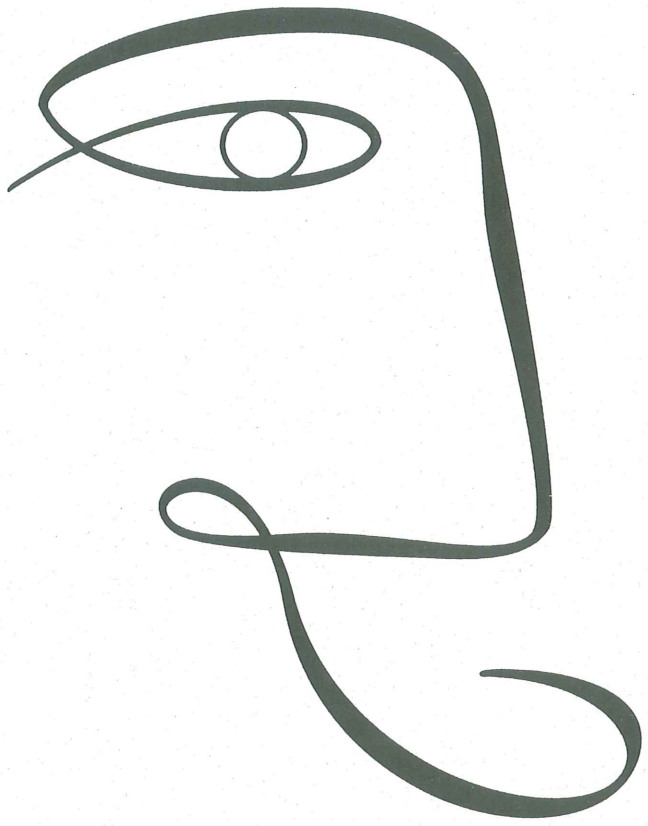


AChemS

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Abstracts

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1 Lecture I. Givaudan Lecture

ANT NAVIGATION: MINI BRAINS - MEGA TASKS - SMART SOLUTIONS

Wehner R. *Department of Zoology, University of Zürich, Zürich, Switzerland*

Ants of the Sahara desert, *Cataglyphis* by name, are skilful navigators. While foraging and homing over distances of several thousand times their body lengths, they accomplish truly formidable tasks. They use a pattern in the sky that is invisible to man to steer their compass courses, and then they integrate all angles steered and all distances covered by remarkable acumen. This system of path integration works even in completely featureless terrain. In addition, *Cataglyphis* can use landmarks by employing photographic skyline memories. Finally, they rely on search strategies that are much more efficient than a random walk would let one assume. The talk focuses on the behavioural performances as well as on the sensory and neural mechanisms that are involved in mediating this behaviour. How can a 0.1-mg brain equipped with a panoramic compound-eye system accomplish these awe-inspiring modes of behaviour? The presentation will focus on the general sensory stratagems employed by *Cataglyphis*, and will show that this small-brain navigator uses simpler tricks than meets the human designer's eye. Cataglyphoid robots are used to test the hypotheses derived from neurophysiological analyses. The general message is that a high-level task can be solved by the co-operation of a number of low-level systems. These low-level systems are adapted to the particular ecological niche, within which the desert navigator operates.

2 Slide II. Gustatory Development

CELL CONTACT-DEPENDENT MECHANISMS SPECIFY TASTE BUD NUMBER EARLY IN EMBRYONIC DEVELOPMENT

Parker M.A.,[1,2] Barlow L.A.[1,2] [1]*Biological Science, Univ. of Denver, Denver, CO;* [2]*Rocky Mountain Taste and Smell Center, UCHSC, Denver, CO*

In amphibians, the ability to make taste buds is an intrinsic property of the oropharyngeal endoderm (Barlow and Northcutt, 1997), implying that within this endoderm mechanisms exist to control cell fate. One possible mechanism for controlling cell fate is intercellular signaling within the epithelium. To determine the timing and mechanisms of cell fate decisions we developed an in vitro assay to disrupt cell contacts within the endoderm. Presumptive oropharyngeal endoderm was explanted from neurula stage axolotls, 9 days before taste buds differentiate. Explanted endoderm was disaggregated, then reaggregated to determine if disruption of normal cell contacts affected the differentiation of taste buds. To assess when cell contacts are important in the development of taste buds, disaggregation and reaggregation (D/R) was performed at progressive developmental stages. Tissue treated immediately following explantation (mid-neurula stage) was unaffected by disruption of normal cell contacts, these D/Rs produce taste buds in numbers comparable to controls. D/R treatment 24 hours later, at early tailbud, however, produced taste buds in much greater numbers than either immediate D/Rs or control explants. Later disaggregation, 36 hours post-explantation, showed a return to normal taste bud number. Our findings imply that there is a critical period in development when cell contact-dependent signals specify the number of developing taste buds. Supported by NIDCD (DC03947 and DC03128 to LAB).

3 Slide II. Gustatory Development

SONIC HEDGEHOG IS AN INHIBITOR OF TASTE PAPILLA DEVELOPMENT

Hall J.M.,[1] Helms J.A.,[2] Finger T.E.[1] [1]*UCHSC, Denver, CO;* [2]*UCSF, San Francisco, CA*

Formation of taste papillae is one of the first steps in taste development. We have shown previously that the developmental signaling molecules Sonic hedgehog (Shh) and the Shh receptor, Patched 1 (Ptc1), as well as Bone Morphogenic Protein 4 (BMP4), are expressed within developing fungiform and circumvallate papillae in mice. Organotypic cultures of embryonic tongues express Shh in a pattern similar to that seen *in vivo*. E11.5 cultured tongues express Shh broadly, but expression localizes to developing papillary regions after 2 days in culture. To determine whether Shh signaling is involved in control of papillary development, we have cultured E11.5 embryonic mouse tongues in the presence of a Shh function-blocking antibody, 5E1. We then assayed for expression of papillary markers *BMP4*, *Ptc1*, and *Shh* using gene-targeted LacZ "knock-in" mice and *in situ* hybridization. Like *Shh*, *BMP4* expression is localized to developing papillary regions after 2 days in culture. In the presence of 5E1 antibody, greater numbers of *BMP4*- and *Shh*-expressing presumptive papillae form. These also are larger than those found in tongues cultured without antibody. *Ptc*, normally expressed evenly throughout the tongue during the culture period, is localized to presumptive papillae in the presence of 5E1. Thus, following exposure to anti-Shh antibody, greater numbers of larger papillae form that more closely recapitulate the *in vivo* pattern of gene expression. These results suggest that Shh acts as an inhibitor of taste papillary formation and growth, a novel role for Shh in developmental signaling.

4 Slide II. Gustatory Development

SHH ACTIVE IN TASTE PAPILLA DEVELOPMENT FROM TONGUE FORMATION THROUGH ADVANCED PAPILLA MOPRHOGENESIS IN EMBRYONIC TONGUE CULTURES

Mistretta C.M.,[1] Gaffield W.,[3] Edwards C.,[2] MacCallum D.K.[2] [1]*School of Dentistry, Univ. Michigan, Ann Arbor, MI;* [2]*Medical School, Univ. Michigan, Ann Arbor, MI;* [3]*ARS, USDA, Albany, CA*

Sonic hedgehog protein (Shh) plays a major role in morphogenesis of epithelial specializations. We previously used the steroidal alkaloid, cyclopamine, to specifically disrupt Shh signaling and subsequently study papilla development and patterning in the embryonic rat tongue. When cyclopamine was added to organ cultures of tongue from embryos at gestational day 14 (E14), fungiform papilla numbers doubled compared to control cultures, and papillae developed in posterior tongue locations where they typically do not form. To learn whether the Shh pathway is also active in papilla development at stages when the tongue has just formed (E13), and much later when papillae are well developed and in full complement on the tongue (E16), we added 5-10 uM cyclopamine to tongue cultures. Tongues were cultured for two days and analyzed with scanning electron microscopy. In E13 cyclopamine - tongues, fungiform papillae were increased in number by about 30% and were also located in more posterior regions, compared to control cultures. Cyclopamine - tongues from E16 embryos had distinctive, diagonal creases with numerous fungiform papillae, giving an impression of surface corrugation. Thus the Shh pathway is involved in papilla and lingual epithelial development and patterning in varying ways, from E13 when the tongue forms, through E16 when papillae have clearly emerged in a patterned array on the well developed tongue. Supported by NIH NIDCD Grant DC00456 to CMM.

5 Slide II. Gustatory Development

AUTODIFFERENTIATION OF TASTE BUDS IN PHARYNGEAL ENDODERM DEPENDS UPON EARLY SIGNALS FROM THE NOTOCHORD

Barlow L.A.[1,2] *[1]Biological Sciences, Univ. of Denver, Denver, CO; [2]Rocky Mountain Taste and Smell Center, UCHSC, Denver, CO*

In amphibians, taste bud development is intrinsic to the oropharyngeal epithelium, which is derived primarily from endoderm. When pharyngeal endoderm is removed from axolotl embryos shortly after gastrulation and cultured alone, the endoderm produces readily recognizable taste buds. This result suggested that pharyngeal endoderm acquires the ability to generate taste buds, i.e., is specified, by signals it encounters during gastrulation. Pharyngeal endoderm sits at the dorsal blastopore lip of the Organizer just before gastrulation. To test if signals from overlying (vertical) and/or adjacent (planar) embryonic regions were necessary for specification, I manipulated pharyngeal endoderm during gastrulation. Regardless of the type of embryological tampering, pharyngeal endoderm generated taste buds, suggesting that specification had occurred before gastrulation. This next idea was tested by explanting pharyngeal endoderm even earlier, before gastrulation. These pre-gastrula endodermal explants failed to produce taste buds. However, when early endoderm was fused with presumptive notochord (another Organizer tissue), the endoderm was now able to generate taste buds. This last finding indicates that signals from notochord within the Organizer, at the onset of gastrulation, induce the autodifferentiative capacity of pharyngeal endoderm. These data further support the notion that the events that give rise to taste buds late in embryogenesis begin very early in development. Supported by NIDCD, DC03128 & DC03947 to LAB.

6 Slide II. Gustatory Development

DEVELOPMENTAL CHANGES IN NEUROTROPHIN RECEPTORS IN GENICULATE GANGLION NEURONS

Rozenblat A., Brann J., Buchholz J.A., Farbman A.I. *Neurobiology & Physiology, Northwestern University, Evanston, IL*

Recent evidence indicates that the neurotrophin, Brain Derived Neurotrophic Factor (BDNF) is expressed in cells of the rat taste bud during development, as early as embryonic day 15 (E15) and in adults. Because BDNF is important for survival of neurons in both the peripheral and central nervous systems we reasoned that its high affinity receptor, one of the trk tyrosine kinases, namely trkB, would be present in neurons of the geniculate ganglion (GG), the sensory neurons that innervate anterior tongue taste buds. Using histological sections of GG that had been immunohistochemically treated to reveal trkA, trkB or trkC, we selectively removed individual neurons and used an RNA amplification method and the polymerase chain reaction (PCR) to examine each neuron for all of the following mRNAs: trkA, trkB, truncated trkB (lacking the tyrosine kinase moiety), trkC and p75 (the low affinity neurotrophin receptor). We used 20-30 cells from each of several developmental ages from E13 to P20. For verification some of the PCR products were subcloned and sequenced. Several embryonic and postnatal neurons expressed either trkA or trkB mRNA alone. Some expressed more than one mRNA, usually trkA and trkB, or trkB and truncated trkB. Truncated trkB mRNA was expressed in more than half of the neurons at all ages. No neurons before age P20 expressed trkC message. A small number at most ages expressed p75. In summary, the patterns of trk expression changed with age in our samples. Correspondence between PCR results and immunohistochemical staining was far less than perfect.

7 Symposium III. Mechanisms of Differentiation and Migration of Progenitor Cells in the Olfactory System

MECHANISMS OF DIFFERENTIATION AND MIGRATION OF OLFACTORY PROGENITORS

Baker H.,[1] Margolis F.L.[2] *[1]Burke Med. Res. Inst., Weill Med. Coll., Cornell Univ., White Plains, NY; [2]Dept. of Anatomy, Univ. Maryland Sch. Med., Baltimore, MD*

A current focus of research in neurobiology is to define how neurons reach their final destination and acquire a differentiated phenotype. The peripheral and central olfactory systems are ideal models for these studies since both show unique migratory behaviors and phenotypic regulation. Frank Margolis will briefly introduce these phenomena in olfactory epithelia. Susan Wray will discuss molecular mechanisms guiding pathfinding and differentiation of placodally-derived GnRH neurons that migrate during early gestation into the CNS. Stewart Anderson will discuss genes required for differentiation of cortical neurons and OB interneuron precursors the latter deriving from the anterior subventricular zone (SVZa) and migrating through the rostral migratory stream (RMS) to populate the olfactory bulb (OB). Aldo Fasolo will describe glial- and neuron-associated molecules in the RMS required for migration of cells to the OB. Marla Luskin will describe stem cell derivation and division in the SVZa and RMS. In summarizing these issues Harriet Baker will focus on phenotype differentiation in OB. In sum, the symposium will present current concepts on a broad range of issues related to olfactory system development. Supported by AG09686 (HB) & DC03112, DC00347 (FM).

8 Symposium III. Mechanisms of Differentiation and Migration of Progenitor Cells in the Olfactory System

MOLECULAR MECHANISMS FOR DIFFERENTIATION AND MIGRATION OF PLACODALLY-DERIVED GNRH NEURONS

Wray S. *Cellular & Developmental Neurobiology, NINDS, NIH, Bethesda, MD*

LHRH neurons, critical for reproduction, are derived from the nasal placode and migrate into the brain along nasal axons. LHRH neurons screened for developmental markers indicate that LHRH neurons diverge from olfactory sensory cells during early stages of nasal placode differentiation. However, LHRH neurons rely on olfactory/vomeronasal axons as their pathway to the CNS. Using single cell cDNA libraries, a novel factor termed nasal embryonic LHRH factor (NELF) was discovered in a differential screen of migrating versus nonmigrating LHRH neurons. NELF is expressed in PNS and CNS tissues, including olfactory sensory cells and LHRH cells in nasal areas. Antisense experiments demonstrated that knockdown of NELF decreased olfactory axon outgrowth and LHRH neuronal migration. These results indicate that NELF plays a role as a common guidance molecule for olfactory axon projections and migration of LHRH cells. NELF is also present at later prenatal stages in the developing cortex and cerebellum, two areas known to exhibit robust neuronal migration, consistent with NELF acting as a migratory signal. We hypothesize that NELF acts via a homophilic interaction and that NELF expression is critical for reproductive function by insuring that LHRH cells reach the developing CNS. Furthermore, downregulation of NELF on LHRH cells as they enter the telencephalon may allow LHRH cells to distinguish a different pathway(s) in the CNS (from those leading to the olfactory bulb and accessory nucleus) and thereby facilitate establishment of the appropriate adult-like LHRH distribution.

9 Symposium III. Mechanisms of Differentiation and Migration of Progenitor Cells in the Olfactory System

DETERMINATION OF CELL FATE WITHIN THE TELENCEPHALON

Anderson S.A., Marin O., Yun K., Long J., Garel S., Rubenstein J. *Dept.of Psychiatry, UCSF, San Francisco, CA*

The telencephalon (basal ganglia, septum, cerebral cortex, and olfactory bulb) contains two general classes of neurons, those that project axons to distant targets, and those that make only local connections. Within these two general classes of neurons there are a myriad of cell subtypes based upon morphology, chemical markers, neurotransmitter, connectivity and physiology. One of the crucial remaining questions about the development of the telencephalon is the molecular determination of neuronal subtypes. Neuronal phenotype can be influenced by factors at various stages of the cell's development. In this talk I will discuss factors which affect cell fate primarily within the telencephalic proliferative zones. Recent evidence has led to the following hypothesis: neurotransmitter phenotype is specified along the dorsal-ventral axis of the neural tube by the combined actions of gradients of signalling molecules interacting with focally expressed transcription factors. Emphasis will be placed upon genes controlling the development of the lateral ganglionic eminence (LGE), which appears to be the source of many olfactory bulb interneurons. Evidence will be presented that the Gsh and Dlx families of transcrption factors play crucial roles in regulating the generation and migration of olfactory bulb interneurons. Interestingly, the secreted signaling molecule Sonic Hedgehog, which has been proposed to regulate cell fate within the LGE, may not be regulating the fate of these cells.

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FACTORS CONTROLLING THE PROLIFERATION AND DIFFERENTIATION OF THE NEURONAL PROGENITOR CELLS IN THE ROSTRAL MIGRATORY STREAM

Luskin M.B. *Cell Biology, Emory University School of Medicine, Atlanta, GA*

An overriding principle of development is that cells become permanently postmitotic once they initiate differentiation. My lab, however, has provided evidence for a population of progenitor cells in rodents and primates that express the properties of differentiated neurons, even though they continue to divide throughout life. These ¬°neuronal progenitor¬± cells are situated in the RMS, a specialized portion of the subventricular zone extending from the anterior tip of the lateral ventricle,a region referred to as the SVZa, to the olfactory bulb (OB). The progeny of SVZa cells migrate to the OB without ever deviating from the RMS. An unusual aspect of SVZa progenitor cells is that even though they have the characteristics of neurons, they undergo division as they migrate. In this symposium I will consider the intrinsic and extrinsic mechanisms that govern the proliferation and differentiation of SVZa neuronal progenitor cells. We have been investigating the roles of cell cycle regulatory proteins, in particular, the cell cycle inhibitor p19INK4d, in the control of SVZa cell proliferation. These studies have suggested that SVZa cells, unlike other progenitor cells, successively exit and re-enter the cell cycle as they migrate. These studies have lead us to analyze whether the Bone Morhogenetic Proteins, known modulators of proliferation, are involved in the regulation of p19INK4d. We are also examining the effects of BDNF on SVZa cell proliferation. These studies have revealed that SVZa cell proliferation is under the control of several interacting factors.

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GLIAL MESHWORK AND CELL MIGRATION IN THE ROSTRAL MIGRATORY STREAM (RMS)

Fasolo A. *Dipartimento di Biologia Animale, University of Turin, Turin, Italy*

Tangential migration characterizes the subependymal layer (SEL) of adult rodents. Chains of migrating cells are ensheathed by a meshwork of astrocytes (glial tubes) up to the olfactory bulb, wherein single neuroblasts spread radially. In the early postnatal period this cell migration occurs in the absence of both chain organization and glial tubes. This suggests that glia in the SEL, rather than being directly involved in cell migration and guidance, could participate in the compartmentalization of a microenvironment subserving cell proliferation at different developmental stages. Analysis of glial- and neuron-associated molecules in the SEL of postnatal and adult rodents reveals that both components express a degree of immaturity. Indeed, cells of the glial tubes, express mature astrocytic markers, as well as the intermediate filament protein vimentin, abundant in radial glial cells. The migrating neuroblasts retain molecules often associated with development as putative regulators of cell migration, e.g. polysialylated NCAM, stathmin, and the neuregulin receptor erbB4. The dipeptide carnosine is found associated with the glial tubes and the neuronal precursors restricted to the tangential part of the pathway. A comparative approach extended to the adult rabbit forebrain shows a very similar pattern of cell proliferation and migration occurring within a different astrocytic arrangement, devoid of well formed glial tubes. These results suggest that cell migration in the mammalian RMS could occur according to different patterns depending on developmental stage and species.

12 Poster IV. Bitter Taste

AMELIORATION OF BITTERNESS AND ORAL SENSATIONS OF CHLORHEXIDINE DIGLUCONATE BY SODIUM GLUCONATE AND SODIUM PROPIONATE SALTS

Portman M.,[1] McConville P.,[1] Alexander S.,[1] Breslin P.,[2] Beauchamp G.[2] [1]*SmithKline Beecham Consumer Healthcare, Weybridge, United Kingdom;* [2]*Monell Chemical Senses Center, Philadelphia, PA*

Aqueous chlorhexidine digluconate (CHX) solutions were perceived as strongly bitter and with associated oral sensations when assessed over a 20 min. period. These oral sensations were the most often identified as tingling' (50%), 'warm/hot' (25%), and 'numbing' (25%). They were of moderate intensity in the mouth and very weak in the throat; differed in magnitude of persistence in mouth and throat and were dose-dependent. The sensations of 'tingling' and 'numbing' tended to persist longer than that of 'warm/hot' (20 min. vs.15 min.). Bitterness persisted for approximately 10 min. at the highest concentration studied. Sodium gluconate and sodium propionate were evaluated for bitterness suppression of CHX. Na-propionate was found to be a more effective suppressor of bitterness and oral sensations when assessed over a 5 min. Na-propionate suppressed the bitterness of a 0.21 %v/v CHX solution by 40% at a concentration of 0.1M compared to a reduction of 30% and 22% for 0.5M and 0.3 M Na-gluconate, respectively. Na-propionate thus offers significant commercial potential in the suppression of bitterness and oral sensations of therapeutic mouthwashes containing CHX.

13 Poster IV. Bitter Taste

A RAPID AND REPRODUCIBLE METHOD TO MEASURE BITTER TASTANT ACTIVATION OF THE GUSTDUCIN/TRANSDUCIN DEPENDENT TASTE TRANSDUCTION PATHWAY

Gravina S.A., McGregor R.A. *Linguagen Corp, Paramus, NJ*

A safe and effective bitter taste blocker would have a substantial impact on the formulation and marketing of many oral pharmaceuticals. The benefits derived from a bitter taste blocker would include increased compliance, especially among the young, removal of high concentrations of sugars from present formulations, and development of new routes of administration by adsorption through the oral mucosa. Our goal is to identify a novel bitter taste blocker by applying recent advances in taste biochemistry to a modern drug discovery program. We have recently developed an assay to measure the activation of the gustducin/transducin dependent taste signal transduction pathway by bitter tastants. The assay is rapid, reproducible and is suitable for conversion to high throughput screening format. Our results show that activation in this assay is dependent on time and the concentration of bitter compounds, and that over 50% of the known bitter compounds tested activate the gustducin/transducin-signaling pathway. Dose dependent studies reveal EC50 values that are related to know *in vivo* potencies of bitter compounds. Furthermore, the maximal *in vitro* responses for bitter compounds varied, suggesting activation of multiple receptor subtypes. The utility of this assay to discover novel bitter taste blockers is demonstrated by the effective inhibition of G protein activation by the known gustducin–dependent bitter blocker adenosine monophosphate.

14 Poster IV. Bitter Taste

TASTE MATCHING AMONG FIVE BITTER COMPOUNDS: CONTINUING STUDIES

Lindsey A.T., Breslin P.A. *Monell Chemical Senses Center, Philadelphia, PA*

We previously reported that moderate concentrations of sugars, acids, and bitter compounds are indiscriminable when their concentrations are adjusted appropriately. Here we present similar forced-choice (duo-trio) experiments to see if matches can be obtained among urea, quinineHCl (QHCl), sucrose octaacetate (SOA), caffeine, and denatonium benzoate (DB). Across sets of trials the concentration of one compound was held (the standard) while the other concentration was varied (the test). We tested urea vs. QHCl, urea vs. SOA at two intensity levels, urea vs. DB, QHCl vs. DB, SOA vs. DB, SOA vs. caffeine, and SOA vs. QHCl. For most subjects, we found concentrations of the test-compounds that could not be discriminated from the standard, P(discrim.)=(0.55/0.50, where the denominator is chance) for 15 discrimination functions. The remaining subjects were able to discriminate among the stimuli more easily, P(discrim.)=(0.67/0.50) for 7 functions. In the latter subjects, discrimination among compounds may have occurred due to temporal and spatial differences in bitterness. At the same concentration of a standard, matches for bitter test stimuli could be up to 2 log units different across subjects. This could reflect individual differences in the type or density of transduction sequences for the five compounds. We surmise that the tastes elicited by these compounds are indistinguishable to most subjects because they act either at a common receptor cell type or at a higher level of signal integration to give rise to indistinguishable neural signals. This research was supported by NIH R29 DC02995 (PASB).

15 Poster IV. Bitter Taste

THE EFFECTS OF IXTH NERVE TRANSECTION ON SHORT-TERM TASTE RESPONSES TO BITTER-TASTING STIMULI IN INBRED AND CONGENIC MICE

Ndubuizu O.,[1,2] St. John S.J.,[1] Smith D.V.,[1] Boughter J.D.[1] [1]*Anatomy & Neurobiology, University of Maryland School of Medicine, Baltimore, MD;* [2]*University of Maryland Baltimore County, Baltimore, MD*

SWR/J (SW) mice are sensitive to a variety of bitter taste stimuli, such as sucrose octaacetate (SOA) and quinine hydrochloride (QHCl). In contrast, C3HeB/FeJ (C3) mice are indifferent to many normally-avoided compounds, particularly SOA. C3.SW-Soa^a congenic taster (T) mice have been developed that are genetically identical to C3 mice except for a small segment of DNA containing the Soa locus, hypothesized to code for a taste receptor. Following 24-hr water deprivation, licking behavior in SW, C3, and T mice to concentrations of SOA (0.00018 – 0.18 mM) and QHCl (0.01 – 1 mM) was compared to water lick rates. SW and C3.SW mice avoided SOA and QHCl as a function of increasing concentration, whereas C3 mice avoided only QHCl. Bilateral transection of cranial nerve IX, which innervates taste buds in the posterior tongue, resulted in a significantly reduced avoidance by SW and T mice, but not C3 mice, of SOA and QHCl in post-surgical tests. Because the avoidance behavior remained largely intact at the higher concentrations, however, we concluded that other taste bud populations (particularly those innervated by cranial nerve VII) play an important role in mediating avoidance of non-preferred taste stimuli. The limited effect of IXth nerve transection on avoidance in mice is surprising given the robust physiological response of this nerve to bitter-tasting compounds. Supported by NIDCD Grant DC00353 to D.V.S., and UMB Grant 01-063 to J.D.B.

16 Poster IV. Bitter Taste

IMPACT OF CHLORHEXIDINE ON HUMAN BITTER TASTE PERCEPTION

Tharp C.D., Breslin P.A. *Monell Chemical Senses Center, Philadelphia, PA*

Previously, we demonstrated that an acute rinse of chlorhexidine (CHX) decreases the saltiness of NaCl, KCl and NH_4Cl, and the bitterness of quinineHCl (QHCl). To determine the generality of its bitterness blocking, we investigated the impact of CHX on the bitterness of $MgSO_4$, urea, denatonium benzoate (DB), QHCl, caffeine, sucrose octaacetate (SOA) and itself. All stimuli were matched for total intensity to facilitate comparisons. There were three separate experimental conditions: (1)intensity matching and time-intensity testing after (2)self-adaptation and (3)CHX pre-rinsing. Self-adaptation and CHX treatments involved two 30 sec rinses. Subjects rated total taste intensity before and during conditions 2 and 3 over a period of three minutes. Relative to self-adaptation, CHX was found to further decrease the bitterness of SOA and caffeine by 36%, QHCl by 30%, DB by 13%, urea by 6%, and $MgSO_4$ bitterness was increased by 37%. The bitterness of CHX was self-suppressed by 31%. CHX was a potent but selective bitter taste suppressor. The effects of CHX were not likely due to cross-adaptation, since it could suppress bitterness beyond the level of self-adaptation. Since CHX's anti-septic activities are due, in part, to its ability to alter membrane bound enzymes, perhaps it also alters selected bitterness transduction proteins. Converging evidence now suggests a link between the taste transduction of SOA and caffeine. Perhaps CHX alters specific bitterness transduction proteins, which decreases the TRC's ability to respond to SOA, caffeine, QHCl, and CHX. This research was supported by grant DC00295-5 to PASB.

17 Poster IV. Bitter Taste

RATS CANNOT BE TRAINED TO DISCRIMINATE QUININE HYDROCHLORIDE FROM DENATONIUM BENZOATE

Kopka S.L., Spector A.C. *Department of Psychology, Center for Taste and Smell, University of Florida, Gainesville, FL*

Little is known about the ability of rats to discriminate between "bitter-tasting" compounds. Reports have shown that receptor sites for diverse "bitter" substances can be co-localized on single taste receptor cells leading to the hypothesis that rats may have difficulty discriminating such compounds. Thus, we attempted to train rats on a two-lever operant task which requires discrimination of two prototypical "bitter" compounds, quinine hydrochloride and denatonium benzoate. We trained a group of rats to press one lever in response to quinine, and another lever in response to denatonium. Even after extensive training, rats did not perform better than chance. This was not due to a general inability to learn the task, since the rats subsequently learned to perform a quinine vs. KCl discrimination task at an average of 90% correct. A positive control group was initially trained on a quinine vs. KCl discrimination task, with mean scores of 90% correct. These rats scored approximately 90% correct on subsequent tasks testing denatonium vs. KCl, and NaCl vs. KCl discriminations. When this group was then tested on a quinine vs. denatonium task, performance fell to chance levels and did not improve. These results suggest that quinine and denatonium, two "bitter" substances, produce indiscriminable gustatory sensations in the rat, supporting the hypothesis emerging from molecular findings. Because many bitter substances exist, studies extending our findings to a broader array of bitter compounds would prove interesting and worthwhile. Supported by R01-DC01628.

18 Poster IV. Bitter Taste

BITTER TASTE TRANSDUCTION INVOLVES CALCIUM INFLUX AS WELL AS CALCIUM RELEASE FROM INTRACELLULAR STORES

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Previous studies have shown that the bitter stimulus denatonium (DN) induces an increase in $[Ca^{2+}]i$ due to Ca^{2+} release from intracellular Ca^{2+} stores (Akabas *et al. Science* 242: 1047-50, 1988, Ogura *et al. J. Neurosci* 17:3580-7, 1997). We report here a new finding that long incubation with DN induces sustained increases in $[Ca^{2+}]i$, which result from Ca^{2+} influx. These results were obtained from taste cells of mudpuppy as well as taste cells of transgenic mice expressing green fluorescent protein (GFP) from the gustducin-promoter. Gustducin is a chemoreceptor cell-specific G protein, which is involved in transduction of bitter and sweet tastes (Wong *et al. Nature* 381:796-800, 1996). In a subset of taste cells in mudpuppy, a long incubation with DN induces oscillatory increases in $[Ca^{2+}]i$. In both mudpuppy and mouse, treatment of taste cells with the Ca^{2+}-ATPase inhibitor thapsigargin also elicited Ca^{2+} influx, suggesting that the Ca^{2+} influx is mediated by store-operated channels. Trp channels have been proposed to serve as store-operated channels in other systems, and recently a trp channel, trp-T, has been identified in gustducin-positive taste cells (Perez *et al.* AChemS 2001). Trp-T may be responsible for the sustained and the oscillatory increases in $[Ca^{2+}]i$ in response to DN. Supported by NIH grants DC00766 and DC00244 to Dr. Sue C. Kinnamon, DC03055 and DC03155 to Dr. Robert F. Margolskee. RFM is an Investigator of the Howard Hughes Medical Institute.

19 Poster IV. Bitter Taste

TASTE CELLS THAT DISCRIMINATE BITTER STIMULI

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Recently, a large family of bitter taste receptors was identified in humans and rodents. Individual bitter receptors appeared to respond selectively to a particular compound but taste cells expressed mRNAs for multiple receptors. This was interpreted that single taste cells respond broadly to several bitter compounds and that bitter stimuli are not distinguishable. However, functional studies suggest that different bitter stimuli can be discriminated from each other. We used Ca^{2+} imaging to measure direct activation of taste cells *in situ* to investigate how bitter taste stimuli are detected in taste buds. Taste cells in foliate slices (rat) were loaded with calcium green dextran and viewed with confocal microscopy. Five representative bitter compounds were applied and Ca^{2+} changes in single cells recorded. Only 18% of all cells tested (69/374) showed responses to the bitter compounds. Responses to cycloheximide (10μM) were observed in 14% of taste cells (51/374); quinine (300μM) in 4.5% (17/374); denatonium (100μM) in 3.7% (14/374); PTC (300μM) in 2.4% (9/374); and SOA (500μM) in 1.6% (6/374). Most bitter-sensitive cells (65%, 45/69) responded to only one compound of the 5 tested. Twenty-six percent (18/69) of the cells responded to 2 stimuli, and only 7% (5/69) responded to more than 2 stimuli. Our results argue against the suggestion that individual bitter-sensitive taste cells respond to a wide range of bitter stimuli. Instead, they indicate that most taste cells may be activated by a limited number of bitter compounds, i.e. individual taste cells can discriminate among bitter stimuli. Supported by NIH/NIDCD grants DC04525 (AC) and DC00374 (SDR).

20 Poster V. Hedonics

ODOR HEDONICS VERSUS ODOR INTENSITY

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This experiment analyzed the relationship between subjective intensity and unpleasantness/pleasantness for pyridine, and phenylethyl alcohol. Detection thresholds were first obtained. Then for each subject (n=12), the threshold concentration, one step below, five steps above, a blank and a strong stimulus were administered in random order 10 times. Subjects determined whether an odor was perceived, and recorded its hedonic rating on a scale from "extremely unpleasant" or "extremely pleasant" to "absolutely indifferent". At the beginning and end of the experiment, each subject also labeled the hedonic scales by placing at the proper position on the scale 5 written hedonic descriptors. Intensity descriptors were placed on individual intensity scales in a similar way by each subject. Equal odor concentration sequences were used for hedonic and intensity scaling. Results revealed that the dependence of correct detection and of subjective hedonic scaling on stimulus intensity differed both for the unpleasant and pleasant stimuli. Weak odors, regardless of pleasantness, were hedonically indifferent to the subjects. Values for independent hedonic and intensity scaling tended to be similar, i.e. individual hedonic judgements on odors were greatly codetermined by the odor intensity perceived. We thank the E. Garfield Foundation for financial support of T.R.

21 Poster V. Hedonics

SENSORY ACCEPTABILITY AND SWEET TASTE QUALITY OF SOFT DRINKS

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Paired preference tests were used to compare four types of commercial soft drinks: a regular and a light version of both an orange and a cola soft drink. The aim of the tests was to determine the preference of the participants being naive towards the nature of the soft drinks, and being aware of the presence of a light version of the soft drink. In each test about 100 men and women (aged 11-80) participated, varying in their habits to drink regular or light soft drinks. The majority of all participants (73-80%) have the habit to drink regular soft drinks. Regular orange soft drinks were not significantly preferred over the light version in the tests. Regular cola soft drinks, on the contrary, were significantly preferred over their light equivalents. The consistencies in responses to the tests were 63-66%. Women were significantly more consistent than men. Furthermore, the awareness of the presence of a light soft drink did not influence the (blind) preferences of the participants. EC Grant PL98-4040 is acknowledged for kind support.

22 Poster V. Hedonics

DIFFERENCES IN JUDGMENT OF UNPLEASANT VS. PLEASANT ODORS

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Affective tone is particularly salient in odor perception. Several studies have shown that electrophysiological (Kline & al., 2000) and psychophysiological (Ehrlichman et al., 1997) patterns induced by odors are different for pleasant and unpleasant ones, and that they activate brain structures differentially (Zald & Pardo, 1997). Those results suggest that pleasant and unpleasant odors are mediated, at least by two different systems. Three studies were aimed at demonstrating the existence of a specific way of processing for unpleasant odors. During experiments, subjects had to perform intensity and hedonic tasks. For the first two experiments, response times were recorded. Results of experiment 1 indicate that unpleasant odors were processed faster than pleasant ones during hedonic judgement, while no difference was observed during intensity judgement. Results were replicated in experiment 2 with odors chosen for their weak trigeminal component. In this case, differences were observed between the right and left nostrils. Experiment 3 focused on withdrawal behavior in odor perception, as revealed by autonomic parameters recording. Results will be discussed in relation with theories of emotion assuming opposed approach and withdrawal behavioral systems. Keywords: emotion, olfaction References: Ehrlichman H. et al. (1997) Psychophysiology, 34, 726-729. Kline, J.P. et al. (2000) Biological Psychology, 52, 241-250. Zald, H. and Pardo, J.V. (1997) Proc. Natl. Acad. Sci. USA, 94, 4119-4124.

23 Poster V. Hedonics

FLAVOR EXPERIENCES DURING FORMULA FEEDING ARE RELATED TO CHILDHOOD PREFERENCES

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As part of a program of research designed to investigate the long-term effects of early feeding experiences, the present study exploited the substantial flavor variation inherent in three classes of commercially available infant formulas- traditional milk-based formulas, formulas based on soy proteins, and those based on hydrolyzed proteins. To this aim, we evaluated the preferences of 4- to 6-year-old children (n=102) who were divided into 3 groups based on the type of formula they were fed as infants. Children played a "taste and smell" game with a wide range of food-related odor qualities including infant formulas, as well as the flavor of milk-based and hydrolysate formulas and differently flavored apple juices. The data revealed that the type of formula that children were fed during infancy influenced their preferences when tested several years later. Children who were fed protein hydrolysate formulas were more likely to prefer the sour-flavored juices, as well as the odor and flavor of formulas, and less likely to make negative facial responses during the taste tests, when compared to children who were fed milk-based formulas. That the effects of differential formula feeding also modified children's food preferences is suggested by the mothers' reports that those fed hydrolysates were significantly more likely to prefer broccoli than were those fed milk formulas. These data are consistent with the hypothesis that early flavor experiences influence subsequent flavor preferences even several years following the experience. Supported by NIH Grant HD37119.

24 Poster V. Hedonics

EFFECT OF DELIVERY ON THE HEDONICS OF COMMON ODORS AND ANDOSTERONE

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A change of perception of olfactory stimuli in reproductive females has been reported to depend on a change of female hormones. Until now the results of different investigators are conflicting. While most studies deal with the change of odor perception, especially threshold, during the menstrual cycle, our study focuses on changes of the hedonic of common odors and andosterone after delivery, a highly unlikely time of reproduction. A total of 73 volunteers with an uncomplicated pregnancy and delivery within the last 10 days were examined by means of a psychophysiological olfactory performance test ("Sniffin' Sticks"). The patients had to perform an identification task, a hedonic and an intensity rating, as well as an andosterone discrimination test. A control group (n=44) was tested for data comparison. The whole sample had a normal olfactory function established by the psychophysical identification testing. The postpartum group did not differ statistically significant in the andosterone perception (46,58% correct discrimination) from controls (50%). The hedonic and intensity judgement revealed a huge standard deviation in both groups without a statistical significance. Within the postpartal group mothers of boys had a better score in the identification test (p=0.05). Our results seem to confirm former investigations that there is no change during the menstrual cycle. The often reported high incidence of changes in odor perception related to the hormonal changes of the pregnancy or postpartal period seems to be anectotical.

25 Poster V. Hedonics

VOLATILES IN THE ENVIRONMENT: PERCEPTION OF GASOLINE ODORS

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The EPA-mandated addition of the oxygenate methyl tertiary-butyl ether (MTBE) to gasoline during winter months has prompted a considerable increase in complaints by individuals reporting that they perceive an unpleasant odor resulting from such fuels due to their higher ether content. Correspondingly, individuals have reported that exposure to MTBE in gasoline has resulted in the onset of acute symptoms such as headaches, nausea, disorientation, fatigue and burning in the upper respiratory tract following refueling. Several studies have shown that exposure to sham or real odors resulted in increased symptom reporting when individuals were told to expect malodors or adverse outcomes, thus raising the possibility that symptom reports following exposure to MTBE are primarily related to adverse reactions to its odor and media influences regarding possible adverse health effects, rather than indicative of genuine health problems. Using a forced-choice task, 8 control subjects (CON) and 6 subjects self-reported as sensitive (SRS) to MTBE were asked to distinguish gasoline without additives from gasoline that contained either MTBE alone (15% v/v) or MTBE plus a masking re-odorant compound. Neither the SRS nor most CON subjects could distinguish odor differences among these three conditions. Thus, whatever role MTBE odor may play in symptom reports, those reporting sensitivity to the oxygenate do not appear to have a unique ability to distinguish its presence based on odor alone.

26 Poster V. Hedonics

FOOD AVERSIONS, FOOD NEOPHOBIA, AND DISGUST: INTERRELATIONS AND GENDER DIFFERENCES

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Considerable understanding for the relation to food is available concerning patients with eating disorders and weight problems, whereas less is known how healthy women and men relate to food, especially regarding certain personality factors. The objectives of the present study was therefore to examine the relation between food aversion, food neophobia, and disgust as well as gender differences in these aspects in healthy subjects (80 women and 80 men). The women and men were comparable with respect to self-reports of allergies, dieting, appetite, food-related sickness, food cravings, diabetes, smoking habits, and chemosensory complaints, whereas women more commonly reported eating disorders (26% vs. 9%) and being vegetarian (24% vs. 10%). Questionnaires were used to assess food aversions (analogous to the Craving Questionnaire), food neophobia (the Food Neophobia Scale) and disgust (the Disgust Scale). The results showed a positive correlation between neophobia and disgust but no relations between either aversions and neophobia or between aversions and disgust. Women, compared to men, more commonly reported aversions (34% vs. 20%) and stronger disgust, whereas no gender differences were obtained for neophobia. The findings support the beliefs of gender differences in various aspects of the relation to food, as well as disgust being involved in the response to novel food, evoking the question for further research why disgust may play this role. Sponsored by grants from the Swedish Council for Research in the Humanities and Social Sciences.

27 Poster V. Hedonics

GENETIC VARIATION IN TASTE: ASSOCIATIONS WITH CREAMY SENSATIONS, PREFERENCE FOR AND INTAKE OF HIGH FAT FOODS

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Bitterness of 6-n-propylthiouracil (PROP) is one marker for genetic variation in taste. Forty-one males and 34 females, who reported low dietary restraint, rated the degree of creaminess and of liking/disliking for 10 sampled, high fat foods as well as bitterness of PROP with the general labeled magnitude scale (Green et al, 1993; Bartoshuk et al, in press). Intake of 40 high fat foods (meats, eggs, cheese, dairy, desserts, condiments) was assessed with a frequency questionnaire. PROP bitterness correlated significantly with creaminess of heavy cream, mayonnaise, cheeses (cream cheese, low sodium and cheddar cheeses) and tended to associate with creaminess of chocolates. There was a PROP by sex interaction on fat food preference. In females, nontasters reported greater liking than did supertasters. Males tended to show the opposite response. Twenty-five of 40 foods formed a statistically reliable group. Non-tasters consumed the group significantly more frequently than did supertasters. The sensory results support previous findings. PROP supertasters can experience greater creaminess because of more fungiform papillae, which carry taste and trigeminal input. The preference findings extend similar results from questionnaire data (Duffy & Bartoshuk, 2000). Sensation and environment may affect the PROP-fat preference relationship. The intake data suggest that PROP tasting may influence consumption of core sources of fat in the diet. (NRICGP/USDA 9603745 and NIH DC00283 supported).

28 Poster V. Hedonics

LIFE-SPAN DEVELOPMENT IN ODOR PERCEPTION: HEDONICS, EDIBILITY AND NAMING

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To investigate life-span development in odor perception, 240 participants were presented with 16 common odorants to categorize as pleasant or unpleasant, edible or inedible, and to name by item, first without and then with four written response alternatives (forced-choice). The age groups of 9-10, 11-12, 13-14, 15-19, 20-39 and 40-59 years were compared, with 20 boys/men and 20 girls/women in each age group. The results showed a distinct, continuous increase in pleasantness categorization across age. The ability to correctly judge edibility did, however, improve only slightly over age with an onset at 13-14 years and reached a plateau at 20-39 years. The ability of naming without available response alternatives increased considerably from age 11-12 years and reached its peak at 20-39 years. Although the general performance in naming was considerably better with than without support of response alternatives, the age-related patterns and effect-sizes were very similar in the two naming conditions. These findings support the notions that (1) odor pleasantness increases with (age-induced) experience with the odorant, (2) the ecologically important ability to determine edibility is relatively well developed in childhood, (3) the ecologically less important ability to name an odorant is not well developed in childhood, irrespective of whether or not support is available. Supported by grants from the Swedish Council for Research in the Humanities and Social Sciences.

29 Poster V. Hedonics

ODOR REINFORCEMENT OF NON-NUTRITIVE SUCKING IN PRETERM INFANTS

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Odors can orient and stimulate newborn feeding. However, the chemosensory experience of tube-fed, premature newborns is relatively restricted. Considering the positive effect of non-nutritive sucking (NNS) on oral feeding skills in this clinical population, we conducted an experiment to determine whether controlled exposure to milk odors increased NNS in two groups of tube-fed, preterm infants who differed in the type of milk they were fed (i.e., breast milk, formula). To this aim, we modified a pacifier to deliver the odor of water and either breast milk or formula contingent on NNS in 18 breast-fed and 13 formula-fed infants, respectively. Each of the two trials lasted ten minutes and occurred during a tube feed. Preliminary analyses revealed that breastfed infants sucked significantly more during exposure to mothers' milk when compared to water ($p<0.05$). Odor- induced NNS could facilitate acquisition of feeding skills by tube-fed newborns. Supported by NIH HD38901 and HD37119.

30 Poster V. Hedonics

FLAVOR PREFERENCES DURING CHILDHOOD

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Although it is well known that children's preference for sweet tastes is universal, very little is known about their preference for sour tastes and how such preferences relate to their liking of actual foods. The present study assessed the preferences of 5- to 7-year-old children for sweetness (0.16-0.93 M sucrose) and sourness (0-0.70 M citric acid) in apple juice and their liking of different types of candies and cereals. We also queried mothers about their own eating habits and the types of fruits and vegetables their child ate during the past week. Preliminary analyses revealed that although there was no sex difference in sweet preferences, girls of this age preferred significantly higher levels of sourness in an apple juice compared to boys. The level of sweetness preferred in either juice or cereals was significantly related to the frequency with which the child ate vegetables during the past week and their mothers' variety seeking behavior as related to food. Supported by NIH Grant HD 37119.

31 Poster V. Hedonics

AMELIORATING AGRICULTURAL ODORS: SENSORY AND ANALYTICAL APPROACHES

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Agricultural odors can create conflicts between farmers and neighbors. We have been investigating the perception of swine odors and their amelioration in the laboratory and in real-life settings by using both chemical analytical and human sensory techniques to explore the complex nature of unpleasant odors emanating from swine slurry (SS). Our latest attempt to ameliorate odor from SS relied upon the addition of additives to the diet and/or to SS. Pigs received a diet high in non-starch polysaccharide (HNSP), low in protein (LP), or HNSP+LP, or with copper chlorophyllin complex (0.1%; CCC used by ostomy patients) or chitosan (1%). SS from pigs on each diet was/was not treated with powdered activated charcoal (PAC). Analytical results indicated that several volatiles that contribute to the characteristic SS malodor, e.g., skatol and ethylphenol, were significantly reduced by addition of PAC as well as some dietary treatments. Results from sensory panels revealed that, regardless of the diet, PAC significantly reduced the perceived intensity and unpleasantness of SS. Diet manipulations also affected SS samples; however, interpretation of these results is not straightforward. In Korea, chitosan is being tested/used to reduce the malodors of SS. In our hands, chitosan did not perform well in this task. To date, the most promising food additive appears to be CCC. Funding was provided by the NIH (DC00298, DC00214) and the Pennsylvania Department of Agriculture (ME448427).

32 Poster V. Hedonics

IMPAIRMENT OF ODOR HEDONICS IN MALE PATIENTS WITH SCHIZOPHRENIA

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Deficits in emotional perception and processing have been described in patients with schizophrenia. Impairments in olfactory perception and processing have also been identified. Olfaction and emotion utilize many of the same central nervous system structures, and similar right hemisphere dominance has been proposed for both functions. Olfactory probes may hold promise in exploring limbic system dysfunction in these patients. Odor intensity and hedonics were assessed using the Suprathreshold Amyl Acetate Odor Intensity and Odor Pleasantness Rating Test in a sample of 23 schizophrenia patients and 26 healthy controls. 100 ml glass sniff bottles containing different concentrations of amyl acetate diluted in USP grade light mineral oil were presented to each subject. Each subject rated the perceived intensity and pleasantness of each stimulus on five-point category scales. Patients and controls did not differ in their intensity ratings of the different concentrations, with weak odors being rated as less intense and increasing odor strength being rated as more intense. Despite the similarity in intensity ratings, controls and female patients showed a parallel change in pleasantness ratings over increasing concentration sets. Male patients, in contrast, did not show any significant changes in pleasantness ratings across these different concentration series. These data suggest a disruption in olfactory-limbic brain regions responsible for attaching hedonic valence to sensory stimuli in male patients with schizophrenia.

33 Poster VI. Spatial and Temporal Processing in Olfaction

STATISTICAL METHOD FOR DETECTING THE ACTIVITY CHANGES OF SPONTANEOUSLY ACTIVE OLFACTORY NEURONS

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The spontaneous activity of specific olfactory receptor neurons can either be unaffected, excited, or suppressed by exposure to particular odorants. To understand the quantitative properties of this complex coding scheme one needs to quantify changes in the spontaneous firing rates of the activity of a large number of olfactory receptor neurons. This problem is particularly arduous because neuronal firing rates are naturally extremely variable and changes in them brief. Statistical means of quantifying changes in the spontaneous firing rates of fish olfactory neurons were developed and implemented as S-PLUS computer program so that this algorithm can be used. The method employs cumulative slope analysis (CSA) and is partly based on the properties of renewal and Poisson stochastic processes. Changes in olfactory receptor cell activity are detected by calculating the cumulative distribution of activity before, and then during odor exposure. Excitation is seen as increase in the slope of the distribution function and suppression as a decrease in slope. Changes in slope are evaluated by calculating the empirical distribution function of the activity olfactory receptor neurons with time using local linear regression. Using estimates of the firing rate before stimulation we determine the interval of expected values, which enables detection of changes in firing rate using a non-parametric statistic. The onset, duration, and amplitude of the response are also determined. This novel method does not require one to make assumptions about the stochastic properties of spontaneous activity.

34 Poster VI. Spatial and Temporal Processing in Olfaction

A MODEL OF NETWORK INTERACTIONS IN THE OLFACTORY BULB

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We have developed a detailed, biologically-realistic model of the mammalian olfactory bulb, incorporating the mitral and granule cells and the dendrodendritic synapses between them. The individual cell models were simplified from detailed compartmental models which had been fitted to experimental data. The amplitudes, time courses and transmission delays of the synapses were obtained from the literature. A simple method for specifying the synaptic connections was adopted, based on the limited experimental data in the literature on the statistics of connections between neurons in the bulb. Both electrical and odor stimulation were modeled. A simple model of olfactory inputs was used which captures some qualitative aspects of odor inputs but which is not necessarily quantitatively accurate. As a test of the model, a series of simulation experiments with electrical stimulation were performed and the results agreed quite closely with published experimental data which were not used in developing the model. This gives confidence that the model is capturing some features of network interactions in the real olfactory bulb. Simulation experiments with 'odor' stimulation were then performed to investigate: (i) how the model response (in terms of synchronization and the spatial distribution of activity) is affected by stimulus intensity; (ii) how the response depends on connectivity parameters; and (iii) whether the network makes it easier to discriminate between similar odor inputs.

35 Poster VI. Spatial and Temporal Processing in Olfaction

IMAGING OF SPATIALLY AND TEMPORALLY DISTRIBUTED ACTIVITY IN THE MOUSE OLFACTORY BULB

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We have recorded odorant-evoked distributed activity in the mouse olfactory bulb using the technique of intrinsic signal imaging. We anesthetized mice, opened windows in their crania, and imaged their olfactory bulbs using a cooled 12-bit camera as we applied odorant stimuli using a computer-controlled olfactometer. The images observed under 630nm light typically consisted of three separable signals: a decrease in reflected light across the entire area of the bulb; a distinct decrease in reflected light localized to several foci; and a later generalized increase in reflected light, concentrated over blood vessels. The locations of the activity foci vary with the odorant applied and are of a size and shape consistent with glomeruli. In addition, we have observed temporal variation of the odorant-specific signals. First, different foci peak with different latencies after stimulus application. Second, we have observed variability in the evoked patterns between applications of the same stimulus. Intrinsic signal imaging is a technique which has been previously used to measure average spatially distributed patterns of activity in the olfactory bulb. Our results suggest the possibility that it might also be used to explore the temporal properties of the signals and to quantify the inherent variability of bulbar responses. *(Supported by grants from NIDCD, ONR and DARPA.).*

36 Poster VI. Spatial and Temporal Processing in Olfaction

SPATIAL REPRESENTATION OF SMALL-MOLECULE ODORANTS IN THE RAT OLFACTORY BULB

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We asked whether very small molecules would be processed in the olfactory brain by using the same strategies used for larger molecules, or whether their small size would produce nonspecific binding and widespread activity in the system. We exposed rats to the smallest molecules containing ketone, carboxylic acid, and alcohol functional groups, and we studied evoked activity patterns in the glomerular layer by using the [^{14}C]2-deoxyglucose method. Acetone, formic acid, and methanol evoked relatively simple patterns of activity that differed significantly from each other. These patterns involved modules previously identified for larger aliphatic odorants possessing the same functional groups. Formic acid activated very anterior dorsal modules previously activated by larger aliphatic acids, and acetone activated dorsal modules previously activated by larger ketones. The data suggest that even very small molecules are processed in much the same way as larger members of their chemical families and that there is no evidence of a broader, nonspecific representation of small molecules in the olfactory bulb. The fact that such simple odorants specifically activate the acid and ketone response modules suggests that functional groups may be the primary determinants of activity in these regions. This project was supported by grant DC-03545 from NIDCD, and by the National Research Service Award MH14599-25 from the University of California, Irvine.

37 Poster VI. Spatial and Temporal Processing in Olfaction

ODORANT-INDUCED PERIPHERAL WAVES MODULATE OLFACTORY BULBAR ACTIVITY

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We previously reported that peripheral waves (PWs) are odorant-induced neural oscillations of synchronized populations of olfactory receptor neurons (ORNs) (Parker et al., J. Neurophysiol. 83:2676-2681, 2000). Our current findings indicate that PWs [28.5±5.6 (s.d.) Hz)] modulate olfactory bulb (OB) neural activity. Cross-correlation analysis of odor-induced OB local field potentials (LFP) and either EOG or ORN neural activity showed that oscillations occurring within the OB and olfactory epithelium are correlated, and that the correlation is absent prior to odorant presentation; however, the major OB LFP frequencies during PW activity are lower (≤ 20Hz) than those observed from ORN recordings. Often during PW activity, the dominant PW frequency occurred in the OB LFP and these field potentials increased in magnitude. Further, PW and OB LFP activity became correlated after the initial 0.5 second of the response which was generally required for the initiation of PWs. Notably, during odor-induced PW activity, PWs and OB LFPs often (73% of 15 preparations) became synchronized, whereas prior to odor application only one case (7%) of synchrony was observed. PWs are hypothesized to function to strengthen the synaptic transfer of olfactory information at specific glomeruli within the OB and to facilitate the synaptic potentiation associated with learning and memory. Supported by NIH DC-03792.

38 Poster VI. Spatial and Temporal Processing in Olfaction

POLYAMINES AS OLFACTORY STIMULI IN GOLDFISH

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Five different classes of naturally-occuring compounds (amino acids, bile salts, nucleotides, prostoglandins & gonadal steroids) are known olfactory stimuli of teleosts. We report here evidence for a sixth class, polyamines (cadaverine, putrescine & spermine), which is essential for cell growth and development. Polyamines are present in large amounts in spoiled food and in decaying matter and possibly indicate the quality of a food source. This report is a comparison of the responses of the olfactory system of the goldfish to both polyamines and amino acids since both classes of chemicals are likely to be involved in food selection. Olfactory (EOG) thresholds to amino acids and polyamines are both in the micromolar range, but EOG cross-adaptation experiments indicated that these classes of odorants bind to independent receptors. Further, polyamines result in significantly larger EOG amplitudes than the more stimulatory amino acids (L-arginine, L-lysine & L-methionine). Preliminary single unit analysis indicates that olfactory bulb (OB) neurons that respond excitedly to an amino acid are suppressed by cadaverine and putrescine. Ongoing studies will determine: (1) whether there is a regional responsiveness in the OB to polyamines, and (2) how goldfish respond behaviorally to polyamines. Supported by NIH DC-03792.

39 Poster VI. Spatial and Temporal Processing in Olfaction

CALCIUM IMAGING OF ODOR-EVOKED INPUT TO THE MOUSE OLFACTORY BULB

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We have loaded mouse olfactory receptor neurons with Calcium Green dextran (Friedrich and Korsching, Neuron 18:737-752), allowing us to image patterns of receptor cell input to dorsal olfactory bulb glomeruli in vivo. Odorant-evoked fluorescence increases were rapid (300 - 800 ms rise-time) and allowed individual glomeruli to be easily distinguished. We characterized the topography, specificity and concentration-dependence of glomerular representations of straight-chained hydrocarbon odorants. Patterns of glomerular input were consistent across animals, and some glomeruli could be identified across animals based on their location and odorant response profiles. Aldehydes, ketones, and acetates maximally activated glomeruli in different regions of the bulb. The specificity of individual glomeruli was variable: some glomeruli distinguished between functional group and carbon-chain length, while others were activated by multiple, structurally dissimilar odorants. However, while glomerular response profiles were not easily predicted based on odorant structure, the response profile of a particular glomerulus was discrete and consistent across animals. Increasing odorant concentration recruited input to additional glomeruli so that even moderate concentrations could evoke input to many glomeruli. However, the pattern of recruitment was also specific to particular glomeruli and consistent across animals. These results demonstrate a powerful new method for characterizing odorant representations in the mammalian olfactory bulb. Supported by NIH NS08437 and DC00378.

40 Poster VI. Spatial and Temporal Processing in Olfaction

THE GOLDFISH OLFACTORY BULB ENCODES PHEROMONE INFORMATION USING AT LEAST TWO SPATIAL MAPS

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It is established that odorants can evoke spatially distinctive patterns of activity across the olfactory bulb (OB), although how this coding scheme relates to complex, biologically-relevant odors (such as pheromones) is not known. The goldfish has a well-described pheromone system making it an ideal model for such a test. Using single-unit recording we recorded from over 100 output neurons distributed across the goldfish OB while exposing fish to 5 different mixtures of biologically-relevant odorants known to stimulate different receptors (sex steroids, bile acids, prostaglandins, nucleotides & amino acids). Although most neurons consistently responded with excitation and/or suppression to more than one odor class (including pheromones; Hanson & Sorensen, this symposium), two basic types of response profiles could be quantified: 'generalists' which responded to at least 2 odor classes in the same manner (with excitation or suppression); and 'specialists' which responded to at least 1 odor class in a distinctive manner. Both pheromone generalists and specialists were identified, and whereas the former were distributed widely, specialist neurons exhibited distinct spatial distributions. For example, a single region specialized for steroid pheromone detection was found in the medial OB. These results suggest that the fish OB encodes pheromones and other natural odors using overlapping maps, one which processes information pertaining to many odor classes, the other specialized for recognition of individual odor classes. NIH/DC03792.

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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 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 (Neuroplex). We also simultaneously recorded the EOG from the olfactory epithelium. 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). In the epithelium we recorded a DC signal and an oscillation whose frequency was similar to the middle and rostral oscillation in the bulb. We applied multiple odor presentations with different inter-stimulus intervals (ISI). The oscillations change their character dramatically when additional odor pulses are applied within a short time period (1-20 sec). The Rostral oscillation does not appear in the response to the second or later pulses. The Caudal oscillation on the other hand, doesn't appear to undergo its period doubling bifurcation, exhibiting only a fast 14 Hz frequency. Middle oscillation on the other hand appears to be unchanged. Our results also indicate that the changes of the response to the second odor stimulus differ, depending whether the same odor or a new odor is presented. If the different odor is presented at the second odor pulse, the changes to the oscillatory response to that odor presentation tend to be longer lasting. Supported by NIH grant NS08437.

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ZONAL EXPRESSION PATTERNS OF OLFACTORY RECEPTORS IN SALAMANDER EPITHELIUM USING GENE SPECIFIC PROBES

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Olfactory receptors (OR) are members of a large gene family ranging from about 100 members in fish to 1000 in rodents, with sequence homologies ranging from 25% to 99%. Most ORs are cloned using degenerate primers derived from transmembrane (TM) regions TMII-TMVII or TMIII-TMVI, yielding sequences specific for the coding region and often exhibiting relatively high homology (>90%) between cDNA clones. Over 30 salamander ORs have been cloned and are expressed in zones in the epithelium oriented along the rostro-caudal axis, with most receptor probes labeling cells restricted to rostral, middle, or caudal regions. Since these probes may lack specificity due to cross hybridization to closely homologous receptor mRNA, we cloned gene specific sequences from the 5' non-coding region of three receptors and compared the distribution of olfactory receptor neurons (ORNs) expressing these ORs in the epithelium. In situ hybridization analyses using coding region and gene specific probes revealed similar patterns for each pair; thus these genes are not highly homologous with other salamander ORs. One clone, AT36, is expressed in the medial one-half of the rostral and middle zones, indicating that the organization of OR expression in salamander epithelium consists of complex patterns of overlapping zones oriented along both the rostro-caudal and medio-lateral axis. Supported by grants from NIDCD (J.S.K.) and NSF (J.E.M.).

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SENSOR RESPONSE KINETICS AS A BASIS FOR CHEMICAL RECOGNITION IN AN ARTIFICIAL OLFACTORY SYSTEM

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We are developing an artificial olfactory system with sufficient sensitivity to detect and discriminate low concentration chemical signatures of buried landmines. In a manner inspired by the extraordinary ability of dogs to detect mines, our device actively draws air samples over an array of differentially sensitive chemical sensors. Sensors responding to a pulse of chemical vapor produce temporal signals that can be modeled by a first-order kinetic process describing the vapor-sensor interaction. This kinetic model is similar to that describing the initial odorant-receptor interaction in the biological olfactory system. A function with two parameters (rate constant and amplitude) accurately describes the sensor temporal signals. Pattern recognition algorithms that use the rate constant parameters from an array of sensors can discriminate chemical signals necessary for the landmine detection task. In controlled laboratory tests, our device can detect low ppb concentrations of TNT and related compounds, as well as the odor signature of an anti-personnel landmine. The device can also readily discriminate these compounds from low concentrations of methanol, which is an interferent for one class of sensor. In addition to rapid response, the processing method supports other properties also found in the biological olfactory system, including recognition of odorants at different concentrations and resilience to sensor degradation and drift. These represent marked improvements over previous methods of signal processing and pattern recognition. Supported by DARPA, ONR, and NIDCD.

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FAILURE TO SEE LARGE PERIPHERAL WAVES DRIVING OLFACTORY BULB OSCILLATIONS IN THE RAT

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Because large peripheral oscillations that may drive olfactory bulb oscillations have been reported in the salamander (Dorries and Kauer J. Neurophysiol, 2000) and catfish (Parker et al. J. Neurophysiol. 2000), we investigated this question in rats. Thirteen rats were anesthetized with urethane (1.5 gm/kg). Odorants were applied with an artificial sniff. An electrode was inserted through the olfactory epithelium into the dorsomedial recess to record electroolfactograms (EOG). Two electrodes were placed on the surface of the olfactory bulb in one dorsal and one ventral position. EOGs with amplitudes up to 15 mV were observed associated with olfactory bulb local field potential oscillations in the range of 20-45 Hz. No oscillatory responses in the EOG record stood out of the noise level of about 200 microvolts. Although we could see significant cross correlation between the two bulb recording sites, there was no substantial cross correlation between the EOG recording site and the bulb activity. If peripheral waves drive the olfactory bulb oscillations in these animals, those peripheral waves must occur in a remote part of the epithelium that we did not sample. Supported by grant number DC00113 from the National Institute for Deafness and Other Communication Disorders.

45 Poster VII. Trigeminal Nerve

TRIGEMINAL DETECTABILITY OF SINGLE CHEMICALS AND MIXTURES

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We have begun a systematic study of the degree of chemosensory interaction in chemical mixtures near their detection threshold. Sensory responses are quantified as stimulus-response (i.e., psychometric) functions extending from chance to virtually perfect detection. Selection of chemicals follows a strategy of physicochemical and structural similarity/dissimilarity. This study focuses on eye irritation and nasal pungency evoked by butyl acetate and toluene presented singly and in binary mixtures of varying proportions. The outcome for eyes and nose showed that mixtures of low detectability (i.e., at or below halfway between chance and perfect detection) display complete agonism, in the sense of dose additivity, compared with the detectability of the single components. In contrast, mixtures of high detectability (i.e., above halfway between chance and perfect detection) display less than complete agonism. Considering the results from previously studied mixtures, and despite methodological differences, the overall outcome suggests that physicochemical and structural differences play a role in degree of trigeminal agonism. Further testing of judiciously chosen additional binary and higher order mixtures will confirm whether a structure-activity model of trigeminal impact based on selected physicochemical parameters, successfully applied to single chemicals, can be extended to mixtures. Supported by grant number DC 02741 from the NIDCD, NIH, and by the Center for Indoor Air Research.

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ORBITAL REFLEX AS INDEX OF NASAL IRRITATION

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The trigeminal nerve mediates various reflexes, including some to irritation from vapors. One expresses itself as twitches of the orbicularis oculi muscle in reaction to nasal stimulation. Does the response show a sensitivity similar to that seen perceptually? Do the twitches increase with level of stimulation? To quantify the muscle response, video images of a set of dots placed a few mm below the lower eyelid were digitally recorded. Using a feature extraction algorithm, the dot movements were registered and used to determine mechanical skin strains in tensorial form, including the principal strain E(max). To establish how E(max) varied with vapor concentration, we tested 12 Ss over the range 3.6 to 4.3 log ppm ethyl acetate. An olfactometer of the Kobal-type introduced vapor into one nostril for 3 sec at a time in coordination with videotaping. Subjects had 3-4 trials per concentration, including blanks, with 2-min intervals between trials. The reflex, captured as skin strain on the lower eyelid, increased with concentration and time. Processing of E(max) values by SDT techniques created psychometric functions for individual subjects. The threshold for the response came within a few percent of that obtained psychophysically, a vapor concentration of 3.9-4.0 log ppm. The results suggest that the surface strain measurements may offer a viable objective index of nasal irritation, potentially useful in the field and in the lab. Supported by NIH grant R01 DC00284.

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NICOTINIC EXCITATION OF NOCICEPTIVE NEURONS IN TRIGEMINAL SUBNUCLEUS CAUDALIS (VC): DESENSITIZATION AND CROSS-DESENSITIZATION OF RESPONSES TO STRONG ACIDS AND SALTS

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We recorded responses of neurons in rat Vc to intraoral nicotine, acids and NaCl for comparison with human studies of oral irritation. In anesthetized rats, single units in superficial dorsomedial Vc responding to noxious stimulation of the tongue were selected. Nicotine (600 mM), 5 M NaCl, citric or pentanoic acid (300 mM) were applied to the tongue by constant flow (0.32 ml/min). With nicotine, Vc firing increased significantly at 6 min followed by a decline to baseline, similar to desensitization of irritation by oral nicotine in humans. Reapplication of nicotine no longer activated Vc neurons, similar to self-desensitization in humans. NaCl evoked a progressive increase in Vc firing over the initial 10 min (sensitization), similar to sensitization in humans. Citric and pentanoic acids evoked similar patterns of sensitization in Vc neurons, paralleling human studies showing sensitization to citric acid. Application of pentanoic acid after nicotine elicited much-reduced Vc responses indicative of cross-desensitization. The response patterns of rat Vc neurons to oral irritants parallel human perceptions of irritation, supporting the use of this model to study the neural mechanisms underlying perception.

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ANIMAL MODEL OF ORAL IRRITATION

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We used a 2-bottle paired-preference paradigm to assess aversion to capsaicin (cap). Rats had access to 2 bottles, one containing cap (0.33, 1, 1.65, 3.3, 33 μM) and the other H2O. Each cap concentration was presented under the following conditions: (a) restricted access (2 hr/d) for 2 d, (b) unrestricted access for 2 d, or (c) unrestricted access for 10 d. The 5 cap concentrations were presented in ascending order with 3 d of H2O-only in between. Conditions (a) and (b) were repeated 3 times with 3 weeks of H2O-only between blocks. Bottles were weighed and positions changed daily. In condition (a) cap consumption decreased linearly with the log of concentration, and was highly reproducible across blocks. In condition (b), cap consumption decreased less and non-linearly with concentration, and showed greater variance across blocks. Rats consumed significantly more 33 than 3.3 μM cap, suggesting desensitization. In condition (c), cap consumption was similar to condition (b) but was more linearly related to log of concentration. At high concentrations, significantly less cap was consumed during days 6-10 than days 1-5, suggesting sensitization or a learning effect. The restricted access condition is superior in assessing cap aversion since it showed less variance and appeared devoid of any desensitizing, sensitizing or training effects of prior cap exposure.

COMPARISON OF NSAID ORO-SENSORY PROFILES: EVIDENCE FOR A PHENYLPROPANOIC ACID PATHWAY AND A MEFENAMIC ACID VANILLOID-LIKE PATHWAY

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Ibuprofen elicits a novel irritation profile characterized by pharyngeal stinging and little mouth irritation (Breslin et al., Chem. Senses, 2001). To determine whether this unusual sensory characteristic is unique to ibuprofen, we investigated the relationships between the chemical structures and the resultant oral and pharyngeal irritation of a few known NSAIDS and irritants (ibuprofen, naproxen, flurbiprofen, acetylsalicylic acid, acetaminophen, mefenamic acid, and capsaicin). The phenylpropanoic acids (ibuprofen, naproxen, and flurbiprofen) were closely related by sensory irritation profile. Unlike the phenylpropanoic acids, mefenamic acid irritated the mouth strongly with a high frequency of reported burning sensations. This profile more closely resembled capsaicin; therefore, a second study was conducted to further investigate the perceptual similarities of the two compounds. Cross-sensitization/desensitization experiments on the tip of the tongue revealed that mefenamic acid was both sensitized and desensitized following capsaicin exposure, and that capsaicin was sensitized but not desensitized following mefenamic acid exposure. These results suggest that mefenamic acid acts through transduction mechanism(s) that partially overlap those of capsaicin and are distinct from the mechanisms of the phenylpropanoic acids. This research was supported by Reckitt and Colman and NIH # DC02995-05 to PASB.

THE EFFECTS OF ACETAZOLAMIDE ON TRIGEMINAL SENSITIVITY TO NICOTINE AND CARBON DIOXIDE

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Acetazolamide is a commonly prescribed anticonvulsant and diuretic, frequently used in the treatment of unlocalized seizures, glaucoma, and altitude sickness. The beneficial effects of this compound are believed to be due to its inhibition of carbonic anhydrase (CA), the ubiquitously present enzyme responsible for the conversion of carbon dioxide to carbonic acid. Reports of sensory side effects by patients undergoing acetazolamide therapy have led to investigations into the possible role of CA in trigeminal chemosensitivity. These reports have been verified by several experiments, including those in which oral trigeminal (Komai and Bryant, 1993, Dessirier et al., 2000.) and nasal trigeminal (unpublished data) responses to carbon dioxide were shown to be inhibited by acetazolamide. In this study, we examined the effects of systemic acetazolamide on nasal trigeminal nerve responses to vapor-phase nicotine (12.5 ppm) and carbon dioxide (50%). Multiunit neural recordings were obtained from the ethmoid nerves of anesthetized Sprague-Dawley rats. Stimuli were delivered to the nares of the rats via a computer controlled air-dilution olfactometer. Acetazolamide (5 mg/kg) selectively decreased nerve response to carbon dioxide, whereas response to nicotine did not significantly change. These results are compatible with previous studies on oral trigeminal chemosensitivity to carbon dioxide, and suggest that similar to the oral cavity, CA-mediated intraepithelial acidification is the basis of nasal trigeminal sensitivity to carbon dioxide.

EFFECT OF TETRACAIN APPLIED INTRANASALLY ON OLFACTORY FUNCTION

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The effect of local anesthesia on olfactory function and on interactions between the trigeminal and olfactory system are not fully understood. We tried to anesthetize trigeminal and olfactory system seperately using tetracain. We examined 20 healthy volunteers psychometrically (Sniffin'Sticks). Moreover, all subjects rated introspectively their smelling ability in general,threshold and discrimination ability on a visual rating scale. Tetracaine was applied either in the area of the middle turbinate and the foramen sphenopalatinum, in the upper part of the nasal cavity or on both locations using a cottonoid. After 15 min the effect of the anesthesia was controlled mechanically and the Sniffin'Sticks test was redone. All volunteers subjectively rated their smelling ability as well as their threshold and discrimination statistically significant worse after tetracain. Nevertheless psychometrically only in threshold testing a statistically significant decrease could be demonstrated in the condition in which tetracain was applied close to the middle turbinate, while both other administrations did not result in a statistically significant effect. Applying locally tetracain at different locations in the nose significantly changed the introspectively rated olfactory ability. After testing only threshold was significantly elevated. The reason for the lack of effect on discrimination might be the fact that it uses highly suprathreshold concentrations. Using this type and application of tetracain complete anosmia could not be achieved.

COMPARISON OF SENSORY EFFECTS OF NICOTINE IN HUMANS FOLLOWING BOTH NASAL STIMULATION AND CIGARETTE SMOKING

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Sensory effects of nicotine in humans are mediated in part by the olfactory and the trigeminal system of the upper respiratory tract. Our aim was to compare objective and subjective sensory effects of nicotine stimulation of the nasal mucosa with those induced by cigarette smoking. Four studies were performed. Negative mucosa potentials (NMPs) and intensity estimations for odor, burning, and stinging sensations were determined following nasal stimulation by olfactometer with nicotine vapor (up to 160 µg/l) in smokers and nonsmokers. Pattern reversal evoked potentials (PREPs) and subjective ratings were determined before and after smoking cigarettes with different nicotine levels (up to 1.56 mg/cig.) and/or menthol in smokers only. After nasal stimulation with nicotine, similar dose dependent increases were observed for NMP amplitudes and intensity estimations. After smoking, increases in PREP amplitude (3x) and impact (2.5x), satisfaction, and liking scores were seen with increasing nicotine delivery. Objective and subjective measurements correlated well. Similar effects for PREP and impact were seen with menthol. A shortening of PREP latency, which is considered indicative of central effects, was seen only with cigarettes containing nicotine. In conclusion, some of the same mechanisms for sensory perception are involved after both nasal stimulation and smoking. This suggests that nasal stimulation by olfactometer may be a suitable model for testing the sensory properties of cigarette smoke.

THE NEGATIVE MUCOSAL POTENTIAL (NMP) DETECTS SMALL VARIATIONS IN STIMULUS CONCENTRATION

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Kobal et. al succeeded in 1985 in recording a pain-related potential from the nasal mucosa. Further experiments characterized the NMP as a specific peripheral trigminal correlate. The aim of the current study was (1) to investigate the sensitivity of the NMP to small variations of stimulus concentration (3% V/V CO2) and (2) to determine the concentration threshold for the generation of NMPs following stimulation with CO2. Fourteen healthy subjects participated in the testing sessions investigating NMP-responses to stimuli of 62, 65, 68 % v/v CO2. Additionally subjective intensity estimates were registered. The analysis of variance revealed a significant influence of the factor concentration on the AUC and on the amplitude of the NMP and on the intensity estimates demonstrating the high sensitivity of the NMP ($F=18.20$, $p<0.001$; $F=36.88$, $p<0.001$, $F=37.5$, $p<0.001$). In thirteen healthy subjects (age: 22-39 years) we determined the threshold for the generation of NMPs and the detetection and pain thresholds using CO2 stimuli with 200 ms duration. The means for the threshold of dection, NMP and pain were significantly different (detection: 20.6±9.6, NMP: 42.6±12.5, pain: 50.4±12.0; MANOVA, $F=19.1$, $p<0.001$). Our results point to the exsistance of a subjective prepain range with activation of trigeminal sensors and the generation of NMPs but without subjective pain sensations.

SOLITARY CHEMORECEPTOR CELLS IN THE NASAL RESPIRATORY EPITHELIUM OF RATS AND MICE

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The G-protein α subunit gustducin originally was found in taste cells, but recently was reported in cells of the gut and nasal epithelium of rats and mice. We examined the distribution of gustducin-ir epithelial cells in whole mounts of the nose. Slender, elongated, immunoreactive cells are present in the respiratory epithelium of the septum and turbinates. Sections through an entire rat head show most of the gustducin-immunoreactive cells anterior to the vomeronasal organ. To investigate whether these cells are receptor cells possibly involved in trigeminal chemoreception, we used ultrastructural studies and double label immunocytochemistry with markers found in trigeminal nerves innervating the nose. Trigeminal nerves that innervate the nasal cavity contact immunoreactive cells. Double labeling with CGRP, PGP 9.5 and acetylated tubulin show nerve fibers either very close to, or often wrapped around, the immunoreactive gustducin cells. In addition, selected gustducin-sensitive cells were processed for electron microscopy and showed characteristics of a receptor cell: an apical process with microvilli extending into the nasal lumen and repeated specialized contacts with nerve fibers. These results indicate that trigeminally innervated solitary chemosensory cells are distributed throughout the rostral part of the nasal cavity. These may serve as the transducing elements for trigeminally mediated reflexes of sneezing and coughing.

SENSITIVITY OF CULTURED TRIGEMINAL NEURONS TO ACIDIC PH AND INNOCUOUS COOLING

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Excursions away from normal tissue pH and temperature are normally guarded against by defensive behavioral and physiological responses. In the trigeminal field, pH- and cooling -sensitive neurons participate in such diverse sensations as cooling of the cornea and upper airways as well as CO_2 in the mouth and nose. Using digital fluorescence imaging of cultured neonatal rat trigeminal neurons to measure intracellular Ca^{2+}, $[Ca^{2+}]_i$, we have begun to characterize the underlying mechanisms of neuronal sensitivity and their distribution. For both cooling (from 37°C to 20°C, at 0.3°C/min) and low pH (pH 6.0), the observed increases in $[Ca^{2+}]_i$ are dependent on external Ca^{2+}. The responses to both low pH and cooling in some neurons are independent of external Na^+ while in others they are strongly dependent. Cool-sensitive neurons are tonically active in vivo, supporting a role for voltage activated channels. L-type calcium channels may be involved in calcium responses to cooling as 10 μM nifedipine inhibited responses. As is found in peripheral nerve recordings, sensitivity to low pH is found in capsaicin-sensitive nociceptors as well as neurons of other modalities. Similarly, sensitivity to innocuous cooling is found to be partially overlapping with sensitivity to cooling.

INCREASES IN INTRACELLULAR CALCIUM TO BOTH METHYL ANTHRANILATE AND CAPSAICIN IN CULTURED CHICK TRIGEMINAL NEURONS

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Behavioral studies have indicated that birds display avoidance behavior to the chemical substance methyl anthranilate (MA). Conversely, birds do not show any aversive behavioral responses to capsaicin (CAP). Using digital fluorescence imaging, cultured chick trigeminal neurons (TG) show an increase in intracellular levels of calcium to both MA and CAP. The object of these experiments was to determine the underlying mechanisms and determine the differences between avian neuronal responses to MA and CAP. We determined the dose-response properties of cultured chick TG neurons to CAP and MA. We tested the response properties over a range of concentrations of MA and CAP from 1 μM to 776 μM. Threshold responses to MA were 10 μM. Chick TG neurons responded to CAP at relatively high concentration (30 μM) compared to mammalian cells (100 nM). Physiologic responses to MA were determined to be dependent on both extracellular of calcium and sodium. Although the responses of these neurons to CAP were also dependent on extracellular calcium, the absence of sodium did not inhibit the calcium responses. This suggests that different mechanisms mediate responses to MA and CAP in the chicken. Supported in part with funding from the USDA, National Wildlife Research Center.

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STUDIES ON COMPOUNDS THAT BLOCK BITTER TASTE

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The ability to detect bitter compounds protects against ingestion of pharmaceutically active and/or poisonous compounds. Most oral dosage pharmaceuticals are bitter and this leads to significant problems of compliance. To rationally address this problem we are studying guanine nucleotide binding regulatory protein (G protein) activation by bitter compounds *in vitro* and *in vivo* to develop effective bitterness blockers. Taste receptor cells contain the taste receptor cell specific G protein gustducin and the closely related G protein transducin. Gustducin has been implicated in the transduction of responses to compounds that humans consider sweet and bitter. A radiometric guanine nucleotide binding assay was developed. In this assay, a receptor activated G protein binds radiolabeled guanosine 5'-O-(3-thiotriphosphate), a non-hydrolysable analogue of guanosine triphosphate, and bound radionucleotide is separated from unbound by filtering. This assay has been scaled to high throughput and we report here the discovery of compounds that inhibit the taste receptor mediated activation of the G protein by bitter compounds. Preliminary *in vivo* data indicate that these compounds do indeed inhibit bitter taste. This high throughput assay has tremendous potential for the pharmaceutical industry as it allows the screening of large libraries of compounds leading to the discovery of bitterness blockers of pharmaceutically active compounds.

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PAPILLAE NUMBER VS. INTENSITY WITHIN & BETWEEN INDIVIDUALS

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Subjects assessed the bitterness of one concentration of 6-n-propyl-2-thiouracil (PROP) and two of quinine HCl presented via filter paper "sandwiches" of our own design. A backing disk (Whatman 1 filter paper, diameter 1.25") provided constant somatosensory stimulation on the tongue and masked the variably sized stimulus filter-paper disk beneath (diameters 0.25, 0.50, 0.75, 1.00, and 1.25"). To prevent diffusion between these disks, a matched-diameter wax paper disk was placed between them. The "sandwich" was placed onto the dorsal-anterior lingual surface, stimulus-disk side down. Subjects rated bitterness of each solution and disk-area combination three times on a labeled magnitude scale. Number of taste papillae stimulated by each sized disk was counted in each individual twice. *Within individuals,* perceived bitterness intensity increased as a function of both stimulation area and papillae number. In contrast, *between individuals,* difference in papillae number was not a good predictor of bitterness intensity. Stimulating approximately the same number of papillae on two different tongues by varying the area of stimulation did not equate bitterness ratings across individuals. Similarly, subjects with many papillae within a fixed area did not necessarily perceive greater bitterness than did subjects with fewer papillae within the same area. These findings suggest that subjects differ not only in their fungiform papillae densities but also in their bitter receptor densities or their bitter transduction mechanisms in a manner that is not easily predicted by papillae density.

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TOTAL BITTER-BLINDNESS: A NOVEL GUSTATORY FAMILIAL TRAIT

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The study of taste genetics has long focused on individual differences in sensitivity to the bitter -N=C-S containing phenylthiocarbamide (PTC) and propylthiouracil (PROP). Here, we introduce a novel bitterness-sensitivity trait carried in a large family that spans five generations. A small proportion of family members possess a total inability to perceive bitterness from a broad range of structurally diverse "bitter-tasting" compounds. Other family members are partially bitter blind; caffeine and sucrose octaacetate (SOA) are not perceived as bitter. Also, many family members perceive bitterness in an ordinary fashion. The trait does not follow simple Mendelian inheritance, nor is it sex-linked. Given the recent report of a large family of bitter receptor genes, it is unlikely that total bitter-blindness is caused by deleterious variation in each bitter receptor gene. In accord with this idea, one bitter-blind family member who donated fungiform gustatory tissue was found to possess mRNA at the predicted sizes for each of eight selected bitter receptors. A common splice component, enabling a single deleterious point mutation to affect all the receptors, is unlikely, since the published bitter-receptor genes are intronless. We are presently sequencing selected receptors and are conducting linkage analyses on this family and another, which carries a similar trait, to identify the gene(s) responsible for absolute bitter blindness. Supported by R29 DC02995 PASB, R03DC50355 & R01DC00082 DRR and DC03969 JGB.

60 Slide VIII. Bitter Taste

IDENTIFICATION OF A NEW SUBFAMILY OF PUTATIVE MAMMALIAN BITTER RECEPTORS

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Recently several members of a putative bitter receptor family (T2Rs) have been isolated (Adler et al., Cell 100, 693-702) and one, mT2R5, has been identified as a receptor for cycloheximide (Chandrashekar et al., Cell 100, 703-11). In order to enlarge our knowledge about the molecular mechanisms underlying bitter perception we isolated and characterized further members of the T2R family. With degenerated primers derived from the published sequences we obtained a fragment of a new putative bitter receptor, rT2RP1, from rat vallate papilla-cDNA. Using this fragment as a probe a phage containing the full length cDNA was isolated. RT-PCR experiments showed rT2RP1 expression in gustatory tissue but not in non-gustatory tongue epithelium. Likewise, rT2RP1 mRNA has not been detected in other tissues, including the brain, liver, heart, spleen, lung, kidney, testis, pancreas, colon, caecum, jejunum and stomach. Within the gustatory epithelium, in situ hybridization revealed the presence of rT2R1 mRNA in vallate papilla taste buds. Analysis of the human genome databases with the rT2RP1 sequence identified a so far unrecognized subfamily of genes and pseudogenes located in two distinct clusters on human chromosome 12. The members of the human T2RP subfamily display sequence identities greater than 81 % among themselves while they have less than 63 % identity to the other known T2Rs. Currently, we are attempting to identify the tastants for the T2RP subfamily by functional expression using the mT2R5 as a positive control.

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VOMERONASAL RECEPTORS IN MOUSE AND HUMAN

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The vomeronasal system of mice is thought to be specialized in the detection of pheromones. Two multigene families (V1r and V2r) have been identified that encode proteins with seven putative transmembrane domains and that are selectively expressed in subsets of neurons of the vomeronasal organ. The products of these vomeronasal receptor (Vr) genes are regarded as candidate pheromone receptors. We have begun to characterize systematically the V1r repertoire in the mouse. We find that V1rs can be arranged into three groups: V1rs of group c are novel and substantially divergent from the other V1rs. Our analysis indicates that the recently described "V3r" genes form a forth group within the V1r repertoire, and do not constitute a novel type of Vr genes. We have also identified a human gene, V1RL1, that encodes a protein homologous to rodents V1rs. We show that a spliced V1RL1 transcript is expressed in the olfactory mucosa of adult humans. Our findings are consistent with a role of this receptor in chemosensation, possibly in the detection of human pheromones.

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THE COMPLETE HUMAN OLFACTORY SUB-GENOME: FROM SEQUENCE TO PHENOTYPE

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Studying the human genome draft with gene mining algorithms, we have identified 906 olfactory receptor putative coding regions (ORs), two thirds of which not previously reported. Of these, 80% are found in clusters of 6-138 genes. The olfactory subgenome encompasses ~1% of the human genome, and ORs are found on all chromosomes except 20 and Y. Chromosome 11 is particularly noticeable, as it contains ~40% of all ORs, suggesting that it is the origin of the subgenome. A large chromosome 11 cluster (~100 ORs) is composed solely of ancient class I 'fish-like' receptors. These have a larger fraction of intact genes, suggesting a functional importance. 531 of the ORs contain frame disruptions, hence are pseudogenes. In addition, for 88 ORs only a partial coding region is known, and their pseudogene status remains undecided. Thus, OR pseudogenes could be as much as two thirds of the total count, suggesting a massive functional gene loss in apes and man. About 70 of the OR pseudogenes have only one frame disruption. Our preliminary results suggest that more than 50% of these potentially recent pseudogenes are polymorphic in the human population, i.e. are functional in some individuals and deleted in others. This widespread genetic variation could underlie the ubiquitous phenomenon of specific anosmias. Using genotype-phenotype correlations, the detailed genetic basis for such chemosensory deficits could be elucidated. Supported by DigiScents, NIH, GIF & Krupp.

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MAMMALIAN TASTE RECEPTORS

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Abstract Not Available

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DROSOPHILA CHEMOSENSORY RECEPTORS

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Abstract Not Available

65 Poster X. Aging

PREVALENCE OF OLFACTORY IMPAIRMENT IN THE YOUNG OLD AND THE OLDEST OLD: RESULTS OF AN EPIDEMIOLOGICAL STUDY

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Although people over age 65 represent the fastest growing segment of the US population, very little is known about how the oldest old differ from the young old. Differences in olfactory function between young adults and the young old have been reported, but there are few data about olfactory function of the oldest old. Given the importance of nutrient intake to maintain good health in old age, this population is of great interest. To directly address this issue, we examined the olfactory function of 2,547 persons, participating in a population-based cohort study, who fell into the following age groups: 50-59, 60-69, 70-79, and 80-97 years. All were individually administered the San Diego Odor Identification Test. The results showed significant progressive loss over the four age groups. Among the young old (52-59) impairment was twice as prevalent among males as among females, with 9.0% of the males impaired versus 4.0% of the females. Among the oldest age group (80-97 years old) 60% of females and 70% of males were impaired. The impact of olfactory impairment on quality of life, safety issues and nutrient intake may be considerable in the oldest old. Supported by NIH grants AG11099 and AG04085 from the National Institute on Aging.

66 Poster X. Aging

REDUCTION OF BRAIN ACTIVATION IN OLD SUBJECTS IN RESPONSE TO ODORANTS DELIVERED IN AQUEOUS SOLUTIONS TO THE MOUTH

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A functional Magnetic Resonance Imaging (fMRI) study was conducted on healthy young and old subjects. Subjects were presented with odorants dissolved in water, delivered to the mouth as boluses of 50 µl every 3 s. Functional images (res: 4x4x4 mm, TR: 4s) were processed with AFNI software. Activated voxels (thr: r>0.42 on the vox, clusters>2 vox) were extracted by correlation to perception profiles and were counted in regions of interest for each subject and each stimulus. Magnitude estimates showed that old subjects perceived the stimuli as significantly less intense than the young subjects did. In young subjects, activation was found in all regions previously described in response to odors presented in air, including primary olfactory areas, orbitofrontal cortex, insula, cingulate gyrus and cerebellum. Significantly fewer activated voxels were found for old subjects in a region including primary olfactory areas, i.e. piriform cortex, hippocampus, amygdala, entorhinal and parahippocampal gyrus. A reduction of activation in elderly subjects in primary olfactory areas is in agreement with a decrease of functionality of medial temporal areas with aging, as well as with psychophysical data indicating impaired performance in olfactory tasks and evoked potential findings of slower processing of olfactory information in the elderly. Supported by NIH grant AG04085 from the National Institute on Aging to CM. We thank Dr. Richard Buxton and Dr. Greg Brown for their expertise.

67 Poster X. Aging

THE INFLUENCE OF AGING ON ODORANT QUALITY PERCEPTION

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We examined odorant quality perception in the elderly independent of any age related shift in perceptual intensity. Investigations were conducted in young (18-30 years) and elderly (65+) normosmic subjects (UPSIT>32/40). The concentration of each of 10 odorants was adjusted separately for each subject with a cross-modal matching paradigm such that all odorants were perceived as being equally intense both within subjects and across subjects. Using these subject specific odorant sets, odorant quality was assessed through ratings of odorant dissimilarity with a labeled magnitude scale (Kurtz et al., 2000). Shifts in odorant quality perception were analyzed by first quantifying how the dissimilarity ratings of each person differed from those of every other person and then placing each person in odorant dissimilarity/people space (Systat, MDS). Young and elderly subjects were located in different locations in this space (GLM, $p < 0.05$) indicating that odorant perceptual relationships were different for the two groups. Odorant quality appears to shift during aging even in people who are outwardly normosmic and in whom any decrement in perceptual intensity is controlled.

68 Poster X. Aging

AGE-RELATED CHANGES IN GENE EXPRESSION IN THE OLFACTORY MUCOSA

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Human psychophysical studies have documented an age-related decline in olfactory sensation. These results correlate well with histologic studies demonstrating a decrease in the area of the olfactory epithelium in older individuals. This process occurs despite the ability of the olfactory epithelium to regenerate neurons throughout adult life. The central question becomes whether this age-related failure of olfactory homeostasis is a result of a decrease in neuronal proliferation or an increase in receptor cell death or both. Ribonuclease protection assays (RPA) were thus employed to analyze changes in expression of pro- and anti-apoptotic genes in the olfactory mucosa of the rat. Genes evaluated included caspase 3, bcl-xl, and bax. Animals were divided into three groups: young (12 weeks), old (24 months), and young bulbectomized rats. Bulbectomized rats were studied at 9 days post surgery, a time when apoptosis and proliferation both occur at accelerated levels. RPA data demonstrate age-related increases in expression of caspase 3, bax and bcl-xl. Bulbectomy triggered a similar pattern of gene expression only greater in magnitude. These findings indicate a relative increase in pro-apoptotic gene expression associated with aging. This pattern of expression is similar to that observed following bulbectomy. These data support the hypothesis that age-related olfactory dysfunction is, at least in part, secondary to an increase in apoptotic neuronal cell death. In short, olfactory receptor neurons generated in older animals may have a relatively greater tendency to enter the apoptotic pathway.

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SENSORY AND COGNITIVE PREDICTORS OF ODOR IDENTIFICATION IN YOUNG AND OLD AGE

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The purpose of this study was to determine predictors of successful odor identification in young and old age. 190 subjects (18-35 years and 69-91 years) were assessed in a number of tasks tapping sensory acuity (i.e., odor sensitivity, intensity discrimination, quality discrimination) and different cognitive domains (i.e., cognitive speed, working memory, verbal fluency). Hierarchical regression analyses revealed that only female sex was a reliable predictor of odor identification in young age. Among older adults, female sex, cognitive speed, and quality discrimination were the most potent predictors of odor identification. Controlling for quality discrimination proficiency and cognitive speed resulted in the effect of age disappearing, indicating the crucial role of these factors for successful odor identification and for age differences in odor identification.

70 Poster X. Aging

A COMPARATIVE STUDY OF THE AGING MOUSE OLFACTORY BULB

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Gene knockout technologies have elevated the mouse as a model in neurobiology. However, relatively little work has examined age and strain differences in the mouse olfactory system. We compared olfactory bulbs from mature and aged (24 month) males of DBA/2, C57BL/6 and BALB/c strains. Only a few differences were observed between strains at 6 months, but both strain and age-related changes were apparent with age. For example, decreases in the volumes of the glomerular (GLM), external plexiform (EPL) and granule cell (GCL) layers were observed, especially in the BALB/c mice. Calretinin staining, observed in the GLM and EPL, was considerably more apparent in the C57BL/6 and DBA/2 varieties than in BALB/c mice. Staining for tyrosine hydroxylase was mostly confined to the periglomerular regions, except in old DBA/2 mice, which exhibited considerable staining in submitral areas. GFAP staining was similar in old BALB/c and DBA/2, with astrocytes in all layers of bulb, but more concentrated in the IPL and GCL. However, in the C57BL/6 tissue very large astrocytes were relatively evenly distributed in all layers. Cell proliferation rates dropped dramatically with age. Labeled cells could be observed along the lateral ventricles, but few were seen within the rostral migratory stream and subventricular zone. Any BrdU immunoreactivity observed within the aged bulbs fit glial cell profiles. Supported by NIDCD grant DC 00338, some aged animals were provided by NIA.

71 Poster X. Aging

FOOD PREFERENCES VARY WITH AGE AND SEX: A NEW ANALYSIS USING THE GENERAL LABELED MAGNITUDE SCALE

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Diet affects disease risk; food preferences in turn affect diet. Using a 9-point scale, Drewnowski et al (1999) showed that preferences for bitter fruits/vegetables increased with age, sweets declined, and high-fat foods did not change. Since the general Labeled Magnitude Scale (LMS; Green et al, 1993; Bartoshuk et al, 2001) has advantages over 9-point scales for sensory measurement, we adapted it for hedonic scaling (Duffy et al, 1999). Preferences for 26 foods were assessed via questionnaire (N=2330); factor analysis produced four food groups showing age effects. Preferences for high-fat foods increased with age, especially for women. Preferences for salty foods increased into adulthood but declined with advancing age. For sweets, preferences declined with age for women but remained stable for men, even though sweet perception is constant across age. These data are consistent with intake measurements from NHANES III (Kant, 2000). Finally, bitter preferences rose with age, presumably since bitter taste perception declines with age. Interestingly, not all food preferences grouped as expected. For example, milk chocolate resembled other sweet foods, but dark chocolate resembled other bitter foods; neither was like other high-fat foods. We conclude that the general LMS both clarifies trends seen with older scaling techniques and reveals new effects that may have health significance. (DC00283 & USDA 9603745).

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THE EFFECT OF AGE ON SNIFFING BEHAVIOR, TASK COMPLETION TIME, AND THE ABILITY TO SELF-IDENTIFY SMELL LOSS

Reinhard T., Dulay M.F., Frank R., Gesteland R. *Psychology, University of Cincinnati, Cincinnati, OH*

Previous research has provided convincing evidence that a decline in olfactory ability exists as a function of age. The goal of the present study was to assess the effect of age on exploratory sniffing behavior in response to a malodor compared to standard measures of olfactory functioning. In addition, the present study explored the effect age has upon the time required to complete different olfactory tasks. Moreover, the capacity of the participant to accurately discern the acuity of her own sense of smell by verbal response was investigated as a function of age. Fifty-three younger adults (mean age = 29 years) and 53 older adults (mean age = 78 years) participated in this study. The olfactory measures employed were the Sniff Magnitude Test (SMT), a two-alternative forced choice butanol threshold task, the UPSIT, and the Alcohol Sniff Test (AST). Results showed that age affects performance on the SMT, with younger adults outperforming the older adults. Similar results were found for the other olfactory tasks. Significant time differences were demonstrated only for the UPSIT and the AST, where older adults performed more slowly. Further, older adults were unable to accurately assess their own sense of smell. Specifically, 83% of the older adults who verbally asserted confidence in a "good" olfactory ability actually performed at the hyposmic or anosmic level. In accordance with the literature, the current study highlights the effect of age on olfactory performance and provides evidence for the validity of the SMT. Supported by NIH DC-4139-01A1 to RG.

73 Poster X. Aging

AGING AND BODY ODOR

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It is commonly believed that many factors can influence odor phenotypes such as an animal's diet, its hormonal status, older people develop a distinct, unpleasant body odor. However, there is little scientific evidence for this and the mechanisms that may underlying this change are not understood. To investigate whether changes body odor with age, we are using mice of different ages as an animal model for aging and odor production. Urine samples were collected from Young (1~3 months old), Adult (3~10 months old) and Aged (>17months old) inbred genetically identical male mice. To study potential changes in odor with age, we tested trained mice in our standard Y-maze assay system. Chemical studies were performed using gas chromatographic analyses of diethyl ether extracts containing volatile, less polar organic chemicals. Urine odors of mice of different ages could be discriminated by trained test mice. In particular, Aged mice smelled different than either Adult or Young mice. From the results of dilution experiments, it was concluded that these differences were due to qualitative rather than quantitative differences in urine odors. The gas chromatographic analysis of urine samples have shown reliable differences related to age. Of the 38 distinguishable gas chromatographic peaks that were reliably quantifiable in either Young, Adult or Aged urine samples, several unidentified peaks differentiated Aged animals. The most prominent differences are increases in 2 peaks in Aged. These results are the first experimental demonstration of an odor associated with aging in a mammal.

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DIFFERENCES IN OLFACTORY ACTIVATION IN REGIONS OF INTEREST IN THE CEREBELLUM OF THE YOUNG AND ELDERLY DETECTED WITH FMRI

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The present study investigated activation of distinct cerebellar regions as a result of olfactory stimulation in healthy young and elderly adults using functional Magnetic Resonance Imaging. Six young and six elderly adults were imaged using a 1.5 Tesla MR scanner. The odorant Amyl Acetate was delivered in 12s ON, 40s OFF cycles. Throughout the scan participants responded with a button press at first detection of each stimulus interval followed by a second button press upon odorant extinction, thus allowing for the creation of an average perception reference vector of the young to which correlation of the MR signal was made. Images were processed with AFNI software. Elderly participants tended to show decreased cerebellar activation in the inferior semilunar lobule, one of three previously identified regions of interest for odor processing, as compared to young adults. Both groups showed similar levels of activation in the superior semilunar lobule. Interestingly, the elderly tended to show more activation than the young do in the third region of interest, the posterior quadrangular lobule. Previous research identifying this area to be involved in attention may suggest the possibility of increased attentional demands placed on elderly adults as a result of decreased olfactory abilities. Supported by NIH grant AG04085 from the National Institute on Aging to CM. We thank Cecelia Kemper, Eman Eghobrial, and Amber Muehlmann.

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COMPOUND-SPECIFIC INDUCTION OF EXTREME SENSITIVITY TO ODORS IN FERTILE WOMEN

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In many species, enhanced olfactory sensitivity of females can facilitate behaviors essential to reproductive fitness, such as predator avoidance, food procurement, mate and offspring recognition. Yet, among humans, studies have failed to demonstrate reliable or consistent gender differences on tests of olfactory sensitivity. In the present study, although women's odor thresholds did not differ from men's initially, women became much more sensitive than men as a function of odor experience. Repeated exposures during threshold tests to a variety of compounds induced dramatic increases in sensitivity among women, but not men. In four studies, absolute detection thresholds dropped an average of 5 orders of magnitude in concentration (range: 3-11 log units) among women. These increases in sensitivity were specific to the induction odorant and/or its odor quality. In two subsequent studies, we explored the role of sex hormones on olfactory sensitization and found that exposure-induced sensitization occurred neither among prepubescent girls or boys nor in post-menopausal women or age-matched men, suggesting that the phenomenon is related to women's reproductive status. Thus, increased sensitivity to weak odorants following repetitive, peri-threshold exposures appears to be a general feature of the olfactory system in hormonally-cycling women. From an evolutionary perspective, odor-induced olfactory sensitization among the reproductive females of a species might confer a number of advantages, especially to the recognition of offspring and pair-bonded mates.

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SNIFF MAGNITUDE AS A CLINICAL MEASURE OF OLFACTORY ACUITY: A COMPARISON TO THRESHOLDS, THE UPSIT AND THE ALCOHOL SNIFF TEST

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A novel approach to the evaluation of olfactory sensitivity has recently been developed that may minimize the impact of cognitive functioning in the assessment of olfaction. The test is based on the reduction of the size and duration of a sniff that occurs in response to a malodor. The task may have an advantage over current clinical measures of olfactory function in that participants are not asked for a verbal response, testing time is short, and the task minimizes the load on attention, working memory, long-term memory, and language skills. The present study assessed the reliability and validity of the new test compared with 3 clinical measures of olfactory function. One hundred and one healthy adults (mean age = 54.7; 36 males, 65 females) were administered the Sniff Magnitude Test (SMT), a two-alternative forced-choice Butanol Threshold Task (BTT), the UPSIT, and the Alcohol Sniff Test (AST) on two different occasions. The test-retest reliability coefficient for each of the tasks was as follows: SMT = 0.75; BTT = 0.69; UPSIT = 0.91; AST = 0.50. The SMT was moderately correlated with all of the standard clinical measures of olfactory function and was found to be highly sensitive and specific in diagnosing anosmia. Results indicate that the SMT is reliable and valid compared to other clinical measures of olfactory function. The moderate correlation among the measures is likely due, in part, to the differential sensitivity of the measures to skills that require higher cognitive processes. Supported by NIH DC-4139-01A1 to RG.

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ODOR DETECTABILITY EXPLICITLY DEFINED AND MEASURED

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Current lack of agreement on key aspects of olfactory function derives largely from doubt over best methods. Uncertainties over intra- and inter-individual variation in odor sensitivity, for example, arise from confusion over what instrumentation, data collection protocol and statistical procedures are most valid. We addressed this issue by developing a comprehensive technique for explicit determination of odor threshold. During each 75-trial session, participants (Ps) were given 15 eight sec clean air (CA) trials interspersed with 15 trials at each of 4 concentrations of n-amyl acetate. After each trial, the P indicated if odor was perceived. The detectability of each stimulus was expressed in terms of the probability (using binomial statistics) that the proportion of detections reported (or a higher proportion) would have been observed had only CA been presented. Concentration ranges were shifted downward over the course of sessions, for each P, so that several sessions were conducted in which the lowest concentration was undetected. Since performance was stable over test days for each P, data from all such sessions were combined in a plot of binomial probability vs. concentration. Logistic regression modeling was then used to fit a single function, with threshold defined as that concentration corresponding to a probability of 0.05. Results from 7 Ps (3 M, 4 F) ranged from 0.08 to 1.14 ppm (v/v). This method may allow accelerated progress in several areas that require valid measurement of odor detectability.

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"MICROSMATIC" PRIMATES REVISITED: OLFACTORY SENSITIVITY IN SQUIRREL MONKEYS AND PIGTAIL MACAQUES

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Primates are typically regarded as visual animals with a poorly developed sense of smell. However, experimental investigations of olfactory performance in primates – with the exception of man – are sparse. Using a conditioning paradigm, we determined olfactory detection thresholds for homologous series of aliphatic esters, alcohols, and carboxylic acids, in two species of nonhuman primates, the squirrel monkey and the pigtail macaque. The results show 1) both primate species to have a well-developed olfactory sensitivity for aliphatic substances, which in some cases matches or even is better than that of species such as the rat or the dog, 2) pigtail macaques to generally perform slightly poorer than the squirrel monkeys, and 3) a negative correlation between perceptibility in terms of olfactory detection thresholds and carbon chain length of the compounds. These findings support the assumption that olfaction may play a significant and hitherto underestimated role in the regulation of primate behavior, and that the concept of primates as primarily visual and "microsmatic" animals needs ot be revised. Supported by the Deutsche Forschungsgemeinschaft (La 635/10-1).

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DIFFERENCE OF AN INDIVIDUAL COMPONENT CONTRIBUTION TO THE INTENSITY OF ODOR IN A MIXTURE

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Inherently, features of odor quality in food flavor are very difficult to comprehend. In general, odor-mixings are not based on a faithfulÅ@summation. Mixture suppression provides a notable example. One of the reasons is the same individual components contribute differently in different contexts. These contributions, which have been touched on from time to time but not fully explored, are the effects of each individual constituent member to the whole odor cocktail. The aim of this study was to examine this contribution. Four components (geranylacetone, 2-pentylfuran, 2-ethyl-1-hexanol and n-valeraldehyde) were nominated for mixture studies. Then, odor intensity matching of these components was performed at the Åemoderate' level on a labeled magnitude scale(LMS). The manner of evaluating the individual component contribution in the neighborhood of 'moderate' was as follows:(I) measuring the change of odor intensity for single component as a function of concentration respectively. (II) Measuring the odor intensity change for mixture when one component in the mixture was increased in concentration. (III) Comparing the change of the intensity of single component(I) withÅ@total intensity change for mixture(II) respectively. In the case of 2-ethyl-1-hexanol and 2-pentylfuran, augmented total intensities were strongly suppressed in mixtures. This contrasted, the intensity change of n-valeraldehyde in mixture was almost same when compared with its single intensity measurement. All these things indicated that compounds have different contributions to the odor intensity when mixed.

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ARE TWO NOSTRILS BETTER THAN ONE?

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This study investigated whether dirhinal olfactory thresholds differ from monorhinal ones, that is, whether bilateral spatial summation occurs at threshold level. Exp. 1 and 2 investigated butanol odor detection thresholds, whereas in Exp. 3 PEA thresholds were assessed. In exp. 2 and 3 Sniffin' Sticks were used for odor presentation, in exp. 1 odors were presented in glass bottles. All participants had normal olfactory function. Thresholds were assessed for the left, the right, and both nostrils in randomized sequences. When comparing results obtained for dirhinal odor presentation to results obtained for the best of the two nostrils, no significant difference was found for any of the three experimental approaches. However, for less sensitive subjects higher thresholds were observed for both nostrils compared to the best nostril. No significant differences were found between the left and right nostrils forany of the experiments. As a rule, female subjects had lower thresholds for both dirhinal testing and their most sensitive nostril when compared tomales. In exp. 3 threshold scores were found to significantly improve when dirhinal testing was repeated. In conclusion the present results indicated that there are no major differences between odor detection thresholds obtained for the best and both nostrils. i.e., bilateral summation does not occur.This may be different for subjects with decreased olfactory sensitivity.

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MALODOR SNIFF WAVEFORM VARIATIONS AMONG SUBJECTS

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Individuals are idiosyncratic in the ways in which they vary their airway pressure in the process of inhaling an air sample through their noses to experience odors (sniff). When they encounter an unpleasant odor (malodor) they become more alike in their behavior, rapidly terminating inhalation in a manner that appears to be mostly reflexive. Olfactory sensibility can be measured quickly and fairly reliably by measuring the extent to which subjects diminish their sniff of a malodor compared to their no-odor or pleasant odor sniffs. This study has as a goal determination of the optimal assay of airway pressure changes to accurately reflect olfactory capability.When a malodor is encountered by surprise, subjects abruptly attempt to diminish the sensation. Inhalation, as measured by peak pressure, pressure fall time, inhalation duration and inhalation volume, is reduced. Sniff pressure rises exponentially, more rapidly in younger subjects than in the elderly. Malodor sniff durations vary among individuals, from 0.3 s to 1.5 s. Fall time is exponential and longer than rise time. Sniff volume in individuals with normal olfactory capability for a malodor is generally about half that of a no-odor sniff. Malodor sniffs are smaller with repeat presentations and in retests. Inhalation volumes for sniffs are at least an order of magnitude larger than respiratory inhalations. Supported by NIH DC-4139-01A1.

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

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Olfaction differs from most sensory systems in having largely ipsilateral afferent projections that bipass the thalamus and extend from the level of the sensory receptors to the primary cortices. Studies of normal and brain-lesioned subjects suggest that the right side of the nose may be superior to the left in some types of olfactory tasks. A number of functional imaging studies find greater odor-induced right than left hemisphere activation. Hence, some degree of right hemisphere "specialization" appears to be present for higher-order olfactory processing. In this preliminary study, we administered a short-term odor memory and discrimination test to each side of the nose of 36 men and 50 women with varying degrees of olfactory ability. We sought to determine, in right-handed subjects, whether (a) short-term odor memory or odor discrimination differs on the two sides of the nose, (b) such differences, if present, are influenced by olfactory ability, and (c) men and women differ on these measures. Odor discrimination, but not odor memory, was found to be better on the right side of the nose of both sexes, regardless of overall olfactory ability. Women, on average, performed better than men on both the memory and discrimination components of the task. These data support the view that odor discrimination is better on the right than on the left. Furthermore, they add further credence to the notion that short-term odor memory does not differ between the two sides of the nose. Supported by NIH Grants PO1 DC 00161, RO1 DC 04278, RO1 DC 02974, and RO1 AG 27496.

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OLFACTORY LEARNING IN YOUNG RATS: DETECTION, DISCRIMINATION AND ACQUISITION OF A REVERSAL LEARNING SET

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A potentially useful method for assessing effects of dietary manipulations in infant rats on learning is to examine acquisition of simple and complex olfactory discrimination problems. To determine feasibility, 11 weaning-aged rats were trained in an olfactometer. Water allowance was yoked to that of controls but training was conducted when rats were 24h thirsty. Correct responses were reinforced with .002ml of 0.1% saccharin. Mean errors (50.9, range 12 – 106) to acquire a simple odor detection task (S+, 5% ethyl acetate; S-, clean air) when approximately 38 days old was slightly but not significantly greater than made by adult rats. A 2-odor discrimination (McCormack food flavorings) was learned quickly (mean errors, 9.6; range, 1 – 48). In 6 subsequent reversals of this task, completed at approximately 46 days of age, mean error scores were 22.0, 23.0, 12.0, 7.4, 6.7, and 2.6, respectively. This performance is also similar to that obtained with adult rats trained on a similar task. Body weights during training did not differ from those of untrained and non-deprived controls. These results demonstrate that very young rats can learn both simple and complex odor discrimination tasks and, hence, are suitable subjects for studying dietary effects on early cognitive behavior.

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AFFECTS OF THE HERBICIDE METOLACHLOR ON PERCEPTION OF CHEMICAL STILMULI BY CRAYFISH, ORCONECTES RUSTICUS

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Previous research has suggested that certain environmental pollutants interfere with the perception of chemical stimuli. It is not clear if the interference lies in masking the odor in the water itself or if there is a physiological effect on the olfactory receptor cells that can influence the behavior of the animal. In this study we exposed crayfish to the agricultural chemical Metolachlor in an acute fashion. We subsequently tested the ability of the crayfish to perceive and respond appropriately to two chemical stimuli important to their survival, detection of damaged conspecifics and location of food resources. Results indicate that the behavior of exposed crayfish toward odor is detrimentally changed. We conclude that there is an affect on the ability of crayfish to perceive chemical signals after being exposed to the chemical Metolachlor and that this affect could alter the ecology of this organism.

SMELL OF MARIJUANA AS PROBABLE CAUSE

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The smell of marijuana (*Cannabis sativa L.*) is commonly used by law enforcement officers as probable cause for entering vehicles and dwellings to search for illicit drugs. However, little quantitative data are available concerning the appropriateness of this action. We present data from two studies based upon legal cases in which the odor of marijuana was used as probable cause for search. In Experiment 1, subjects were first found to be able to discern, at point blank range, the odor of ~ 2.5 kg of processed marijuana packaged in a closed plastic bag and discriminate between this odor and the odor eminating from a plastic bag that contained the same weight of bailed newspaper. A similar determination could not be made, however, when smelling occurred in the passenger's compartment of an automobile whose trunk contained either the same packaged marijuana or newspaper. In Experiment 2, the odor of immature (non-budding) marijuana plants was found, using the method of magnitude estimation, to be generally weaker than that of mature (budding) marijuana plants. An empirical study employing an olfactometer revealed that subjects were unable to discern the odor of volatiles from immature marijuana plants when they were mixed with diesel exhaust fumes in a ratio modeled from the chimney effluence of a real-life growing situation in an illicit California grow room. Although these studies represent the first to examine the ability of humans to detect marijuana in situations encountered by law enforcement officers, additional studies are needed to determine the degree to which their findings generalize to similar situations.

GENERALIZATION OF CONDITIONED FEEDING RESPONSE TO ODORS REVEALS OVERLAPPING ODOR REPRESENTATIONS IN THE MOTH, *MANDUCA SEXTA*

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Olfactory systems can detect and discriminate among a wide array of monomolecular odourants and blends. Given the limited number of neurons used to code such a diverse stimulus domain, neural representations for odours must overlap in a cross-fiber coding scheme. Here we use the generalization of a conditioned feeding response in the sphinx moth, *Manduca sexta*, to quantify three geometry-based dimensions of odour space in which monomolecular odours may be assessed. We show that generalization of a feeding response from a conditioned monomolecular odour to another is a function of the length and shape of the carbon chain and the functional group on the molecule. Moths conditioned to 2-hexanone or 1-decanol and tested with a number of alcohols and ketones exhibited decreased response probabilities, as a function of the chain length and functional group. In contrast, when conditioned to 1-hexanol moths failed to distinguish alcohols from ketones. In all cases chain length did and functional group did not interact indicating the independence of these dimensions. Differential conditioning of alcohols and ketones revealed interaction of excitatory and inhibitory generalization gradients within an odour dimension. When odourants were distinct to the moths, the existence of an inhibitory gradient shifted the peak excitatory response away from the locus of inhibition. These data substantiate the claim that these are relevant coding dimensions within a cross-fiber coding scheme whereby odours are coded by spatiotemporally overlapping sets of neurons.

A COMPARATIVE IMMUNOCYTOCHEMICAL STUDY OF THE RAT VOMERONASAL SYSTEM IN DEVELOPMENT AND REGENERATION AFTER NERVE TRANSECTION

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Vomeronasal neurons express high levels of neuronal plasticity during development and after injury. We used immunocytochemical methods to compare stages of vomeronasal organ and accessory olfactory bulb development to those of regeneration following nerve transection. At E15 and at six to ten days after injury, nestin positive cells were observed within the sensory epithelium. The early appearance of nestin positive cells suggests that they represent precursor cells. N-CAM positive cells begin to appear 8 days after injury. OMP and N-CAM double positive neurons were observed in the mature adult epithelium and to a lesser extent after 10-60 days of recovery from nerve transection. Axon projections to the accessory olfactory bulb did not recover following nerve transection. This could explain the absence of mature vomeronasal neurons (OMP positive cells) in the sensory epithelium. The ability to identify and classify cells based on immunocytochemical markers may prove useful in future studies of neurogenesis and regeneration in chemosensory systems.

NEUROGENESIS OF RAT VOMERONASAL NEURONS *IN VITRO*

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The vomeronasal system has been regarded as an ideal model for the study of neurogenesis, regeneration, axonal guidance, and development of neurons in the mammalian nervous system. Vomeronasal neurons undergo continuous neurogenesis throughout the life span of most animals. Precursor cells have the ability to undergo cell division and differentiate into neurons. Previous studies have shown that partially dissociated VNO cells when cultured on the feeder layer formed vomeronasal epithelium-like structures called vomeronasal-pocket and continuous degeneration and regeneration of axon bundles were observed. vomeronasal-pocket contained both vomeronasal neurons and supporting cells. They formed a spherical structure with a central cavity where microvilli protruded from supporting cells. In the present study, we conducted immunocytochemical analysis to identify cell types of vomeronasal-pocket and to examine developmental stages of vomeronasal neurons *in vitro*. We also performed nerve transection of vomeronasal-pocket cells and examined neurogenesis of vomeronasal neurons. The culture system may provide an important tool for future studies to examine the mechanisms underlying neurogenesis and differentiation of vomeronasal neurons.

LIMITS AND POTENTIAL OF BASAL CELL TRANSPLANTATION

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The olfactory epithelium (OE) supports neurogenesis throughout life and reconstitutes both neuronal and nonneuronal cells after direct experimental injury. We are using a colony forming unit (CFU)-assay, based on transplantation into MeBr-lesioned host OE, to elucidate the functional capacity of defined types of OE cells. As a first step in assaying the limits on the CFU-approach, we performed two sets of experiments: (1) transplantation of cells dissociated from OMP-LacZ transgenic mouse OE; (2) transplantation of nls-LacZ-RRVV-labeled dissociated cells from adult rat OE after removing NCAM (+) cells. Data from both studies showed that transplanted basal cells could survive, integrate and mature in the host OE. Subsequent studies assessed the capacity of specific types of OE cells. We FACS-isolated horizontal basal cells (HBCs) using BS-I lectin and globose basal cells (GBCs) using GBC-2 Ab, labeled them with fluorescent latex microspheres or nls-LacZ-RRVV, and transplanted them. Transplanted GBCs gave rise to both HBCs and GBCs while transplanted HBCs gave rise to only HBCs, confirming that GBCs are multipotent in this milieu and indicating that HBCs are not. We also performed transplants using a conditionally-immortalized, basal cell-derived line, Odora-B. After engraftment, the Odora cells seemed to differentiate normally. Collectively, our data suggest that transplantation can be used to elucidate the mechanisms underlying the reconstitution the OE after lesion and may provide a therapeutic approach for human dysosmia. Supported by DC02167.

DEVELOPMENTAL CHANGES OF SEROTONIN RECEPTOR 5-HT3 SUBUNIT mRNAS IN THE RAT OLFACTORY EPITHELIUM: INVOLVEMENT IN NEUROGENESIS?

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The mammalian olfactory epithelium is characterized by neurogenesis throughout life. Pro-liferation density decreases postnatally, however, cellular and molecular mechanisms underlying this regulation are poorly understood. Serotonin has been implicated in the neurogenesis of the central nervous system. Its actions are mediated by multiple receptor subtypes. The pattern of the 5-HT3 receptor mRNA expression within the brain suggests possible roles for its involvement in proliferation. Using RT-PCR we demonstrated the expression of 5-HT3 receptor subunit mRNAs in the olfactory mucosa of postnatal rats at different ages. Semiquantitative analysis of the PCR-products revealed a marked decrease in the expression of 5-HT3 receptor alpha-subunit mRNA with increasing age of the animals. Animals at postnatal day 10 (P10) had about 4 times the amount of mRNA of P36, who had 4 times that of P450. The results of the tested age groups P98 and P120 fitted into this pattern. The expression of the 5-HT3 receptor beta-subunit mRNA changes in a nearly opposite direction. Whereas P10 showed a low signal, the juvenile and adult rats had increased amounts of beta-subunit mRNA. Very old rats revealed again less PCR-products. The expression of other serotonin receptors, such as 5-HT2B did not change postnatally. The decrease in 5-HT3 receptor alpha-subunit mRNA parallels the decrease in proliferation density in the olfactory epithelium of postnatal rats. In further studies we will investigate the functional role of the 5-HT3 receptor in neurogenesis.

FUNCTIONAL ANALYSIS OF *XDLL3* IN OLFACTORY SYSTEM DEVELOPMENT

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Distal-less genes are transcription factors. One *Distal-less* gene in *Xenopus laevis*, *Xdll3* (homolog to mouse *Dlx5*), is expressed in cells fated to become olfactory placodes and olfactory bulbs. In early embryos, *Xdll3* is expressed throughout the sense plate; later, *Xdll3* expression becomes restricted to the olfactory placodes. A few stages later, *Xdll3* is expressed in the presumptive olfactory bulb (Franco et al., submitted). Based on the early expression pattern of *Xdll3*, we hypothesized that overexpression of this gene might result in an increase in the size of the olfactory placode and bulb. To test this, *Xdll3* mRNA, along with *βgal* mRNA as a marker, was injected into one cell of a two-celled embryo, and embryos and young larval animals were processed for *Bgal* staining and morphological analysis. Control animals received only injections of *βgal* mRNA. Our experiments show that in a subpopulation of animals injected with *Xdll3*, the nasal capsule and olfactory bulb on the injection side are larger. Remarkably, the eye on the injected side appears to be reduced. Potential mechanisms for these results might be (1) cells fated to become retina are shifted to olfactory structures or (2) *Xdll3* overexpression causes proliferation of cells in the olfactory placode and bulb, while in the retina, it has an inhibitory effect on *Xdll4* function (*Xdll3* is not expressed in the eye). In summary, our current experiments are designed to quantify the expansion of olfactory structures and the reduction of the eye. Support: NINDS#NS37147 and Beckman Foundation.

TRANSCRIPTS ENRICHED IN THE PROLIFERATION ZONE OF THE LOBSTER OLFACTORY ORGAN

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We are investigating development-related differences in gene expression in the olfactory organ (antennule) of the spiny lobster *Panulirus argus*. The antennule consists of repeated segments, some of which contain aesthetasc sensilla innervated by olfactory receptor neurons (ORNs). The ages of ORNs range linearly from youngest in the proximal proliferation zone to the oldest in the distal senescence zone (Steullet et al., 2000, J. Neurosci. 20: 3282). We hypothesize that many transcripts enriched in the proliferation zone will be involved in genesis of ORNs and their precursors, in cell differentiation, or in aesthetasc formation. We used representational difference analysis (RDA) to amplify cDNA fragments more abundant in the proliferation zone than in the mature zone in the middle of the antennule. Cloned fragments contained at least 25 distinct sequences, 11 of which are novel. The others were a monooxygenase, two 11.5 KDa antibacterial proteins, 4 cuticle proteins, a collagen α-1 (II) chain precursor, a trypsin-like serine protease, an embryonic serine protease, a serine protease inhibitor, a TGF-β inhibitor, and a member of the CCN (CTGF/CR 61/Nov) family of growth factors. We are performing RNA dot blots to confirm that these clones are more abundant in the proliferation zone than in the mature zone. If confirmed, these results suggest that the CCN and TGF-β signaling pathways might regulate olfactory neurogenesis in the spiny lobster. Supported by NIH DC00312 and NIH DC02366.

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CELL DYNAMICS IN THE SEPTAL ORGAN OF MASERA

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The rat septal organ (SO), also called the organ of Masera, is an isolated patch of olfactory epithelium in the ventral region of the nasal septum. We asked if neural replacement in the rat SO is the same as that in the olfactory epithelium proper (OE) during postnatal development. Area measurements and proliferation density (number of BrdU-positive cells/mm length) were evaluated in coronal histological sections of rat heads from newborns (P1) to 11 month-old animals (P333). In newborn rats (P1) the SO is separated from the OE by 0.36 mm of respiratory epithelium. During postnatal development the SO "moves" further apart, about 2.8 mm from OE in adults. The surface area of the SO increases continuously from P1 (0.15 sq mm) to P105 (1.75 sq mm) and then decreases (P181, 1.25 sq mm), whereas the area of OE in the rest of the nasal cavity continues to increase at least up to one year. Proliferation density of basal cells in the SO decreases from 110/mm at P1 to about 14.6/mm at P105 and then remains constant. Thus, although the SO area declines the proliferation density remains the same. In contrast, the OE proliferation density is higher at P1 than in the SO, and continues to decline after P105. A possible explanation for the differences in cell dynamics between SO and OE is that neurons in the SO are more vulnerable to damage because of their location, in the anterior ventral region of the nasal septum and near the nasopalatine duct. The surface area declines after P105, indicating the SO is less able to maintain itself by cell replacement, even though the proliferation density remains constant.

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GENES UP-REGULATED AT METAMORPHOSIS IN *XENOPUS LAEVIS* NASAL CAPSULES

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The clawed frog, *Xenopus laevis*, is a suitable model to study the mechanisms of thyroid hormone regulation of olfactory epithelium remodeling at metamorphosis. We have shown that thyroid hormone stimulates numerous morphological and biochemical changes in the olfactory system at metamorphosis (Burd, 1999). The goal of the present study was to identify genes involved in olfactory system remodeling at metamorphosis. Using the Clontech PCR-Select Subtractive Hybridization kit, first strand synthesis of polyA RNA from metamorphic climax (stage 58-60) versus pre-metamorphic (stage 40-47) tadpole noses was generated, amplified and subtracted to enrich for metamorphic climax expressed genes. The final, subtractive hybridization pool was ligated using a TA-cloning vector with blue/white screening. Seventy-four clones were initially screened for inserts using nested PCR primers from the subtraction kit. Forty-three had inserts and were sequenced: 13 contained homopolymers, 17 had minimal homology to BLAST database sequences, 13 others were considered good hits, and 7 of the 13 matched *Xenopus* database sequences. *Aldehyde dehydrogenase I* (*ALDH1*), *zinc finger protein* (*XFG 5-1*), and *calbindin* were selected for analysis with semi-quantitative RT-PCR. *ALDH1* and *XFG 5-1* showed elevated expression during metamorphosis; *calbindin* was not detected in the pre-metamorphosis tissue sample, but was expressed during metamorphosis. This analysis supports stage related expression levels for these three genes. Support: NIDCD #DC03905.

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VITAMIN A DEFICIENCY LEADS TO INCREASED CELL PROLIFERATION IN OLFACTORY EPITHELIUM OF MATURE RATS

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We have shown that Vitamin A deficiency (VAD) reduces expression of two different markers of mature olfactory neurons (ONS) in mature rats, suggesting a lack of VA derivatives, such as retinoic acid (RA), blocks differentiation of ONS. We note no gross degeneration of VAD tissue and observe increased cell numbers in the basal region of some VAD OE. We examined the possibility that proliferation is upregulated in VAD tissue using two different approaches. (1) We compared total cell number and organization of olfactory epithelia lining VAS and VAD nasal septa and (2) we determined the relative expression levels of mRNA encoding MASH I. The total number of cells in VAS and VAD epithelia were generally comparable, but some basal regions of VAD tissue appeared neoplastic and disorganized. Previous studies have shown that stem cells cultured in the presence of RA are induced to differentiate into neuron-like cells and that MASH I is strongly upregulated by RA. We were surprised to find that MASH I mRNA levels increased in VAD samples. We suggest that MASH I, in this case, is a marker of proliferating, pro-neural cells, and that its increased expression in VAD tissue is the consequence of increased proliferation of MASH I positive pro-neural cells. We conclude that reduced availability of RA in vivo leads to loss of control over proliferation, neoplasia, and increased numbers of pro-neural cells that are blocked from progressing to maturity.

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IS CHIN-MARKING IN THE RABBIT MEDIATED BY THE ACCESSORY OLFACTORY SYSTEM?

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Rabbits of both sexes possess chin glands which they use to mark their surroundings. The frequency of spontaneous chinning and overmarking depends on hormonal state. This readily quantifiable behavior provides an ideal opportunity to study a controversial issue in mammalian olfaction - the extent to which chemical signals are mediated by the accessory olfactory system. Sixteen stud males were tested as follows: 1) Spontaneous chinning. For 3 d, 10 min/d animals were tested in an arena with 3 bricks, and the number of marks directed to these recorded. 2) Chinning elicited by male marks. For 5 d animals were tested after one brick had been marked by a stud male. 3) Chinning elicited by female marks. Steps 1 and 2 were repeated except that in 2 one brick had been marked by an estrous female. 4) Surgery. In n=8 animals bilateral medial lesions of the olfactory bulb were made to destroy the vomeronasal nerve, and n=8 given lateral control lesions. 5) Post-surgical testing. One week later, steps 1-3 were repeated. 6) Histology. Lesions were verified using Nissl-stained sections. Animals showed stable pre-surgical levels of spontaneous chinning and directed more marks to bricks previously marked by conspecifics. Whereas surgery had little effect on spontaneous chinning in either group, medially lesioned animals no longer discriminated between marked and unmarked bricks. Thus, in (male) rabbits the response to chemical signals presumably contained in chin-gland secretion appears to be mediated by the accessory olfactory system.

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ATTRACTION OF CONSPECIFICS TO MOLTED ODOR SIGNALS IN CRAYFISH (*ORCONECTES RUSTICUS*)

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For many organisms olfaction plays a key role in communication. Crayfish use olfactory signals to find mates, warn of predators, and relay social status. While many of these situations have been studied in detail, behavior of conspecifics toward molted individuals has not. The physiology of crayfish changes dramatically during molting (ecdysis), which in turn changes both the content and concentrations of the chemical cues released into the water. We hypothesized that conspecifics would pick up these changes in chemicals released and move toward the molt signal. A Y-maze was used to test for a differential attraction to various odors presented to intermolt (non-molting) crayfish. All crayfish used were of the species *Orconectes rusticus*. The odor sources were molt crayfish, intermolt crayfish, aged tank water (control), or food (fish carrion). Variables measured included initial arm choice, time spent in each arm, and time spent at each nozzle. On average, crayfish spent more time in the presence of molt signals when paired with intermolt or control stimuli. The food stimulus was more attractive over any of the other stimuli. These results demonstrate that there is a difference in the way a conspecific perceives a recently molted individual versus an intermolt individual. Since cannibalism and aggressiveness toward molted individuals has been observed in laboratory situations, the ability of a conspecific to recognize a molted individual by olfaction has implications for both social interactions and survival of individuals in crayfish populations.

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PREDATOR ODORS AND REPRODUCTION IN RODENTS

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We examined the influence of predator odors on reproductive output of rodents. Laboratory naive animals responded to predator chemical cues with reduced litter size and skewed sex ratio. Our studies indicated that exposure to predator urine maximally affected implantation and maintenance of implantation when predator urine was applied to the bedding of rodents (mice and rats) during the first third of gestation. 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.Subsequently, we discovered that the reduction in litter sizes in rodents exposed to predator urine was attributable to suppressed progesterone levels affecting implantation of embryos and not to stress or fear. Chronically high corticosterone levels did not suppress reproductive output.It is noteworthy that suppression of rodent reproduction also occured when rodents were exposed to urine of conspecifics housed under high population densities. The evolutionary adaptive response for reduced litter size is for producing high quality offspring in an environment where food resources are scarce.The fact that rodents respond to certain chemical signals in predator urine in a similar fashion may be fortuitous, and may have more to do with the coincidence that the urine contain similar chemical cues resulting from protein digestion in carnivores and protein catabolism in nutritionally deprived rodents rather than specific predator-prey adaptations.

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MOLECULAR ANALYSIS OF OLFACTION AND HOST PREFERENCE IN THE MALARIA VECTOR MOSQUITO *ANOPHELES GAMBIAE*

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Olfaction significantly influences the behaviors of many medically important insects such as the Afrotropical mosquito *Anopheles gambiae,* which is the principal vector for human malaria. A large measure of this mosquito's vectorial capacity is due to its anthropophilic host choice. Because this preference is mainly directed by chemosensory cues, it is crucial to understand the mechanisms behind olfaction for the design of novel disease control strategies. Our lab is interested in the molecular characterization of mosquito olfaction and host preference. To date, we have focused on gene-discovery projects and have identified several antennal-enriched genes, including odorant binding proteins and arrestins. Analysis of a novel *A. gambiae* arrestin (AgArrH1) has demonstrated expression in both olfactory and visual tissues, suggesting dual function across these sensory modalities. As many olfactory-driven behaviors exhibit circadian fluctuation, it is hypothesized that genes controlling these actions will be influenced by the central pacemaker. Data will be presented examining the potential regulation of olfactory genes by the circadian clock. At the end of the day, we expect that these studies, along with biochemical and functional analyses of the mosquito olfactory system, could contribute to a better understanding of vector-host interactions and provide methods for disrupting the transmission of diseases such as malaria, dengue, and West Nile encephalitis.

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PRELIMINARY EVIDENCE FOR CANINE OLFACTORY DETECTION OF MELANOMA

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Since chemical markers of melanoma have been reported in blood and urine, volatile chemicals might be emitted from melanoma cells (on the skin surface) in amounts sufficient to allow accelerated diagnosis. Using methods normally used in canine olfactory detection of drugs and explosives, two dogs were trained to retrieve hidden tubes containing melanoma tissue samples. Above-chance localization persisted in tests requiring the dog to indicate which box compartment, test tube or location on the body of healthy volunteers held the melanoma target; various distractor stimuli were used. In searches of seven dermatology patients, canine olfactory searches "confirmed" two prior diagnoses. In two additional patients, definitive indications by one or both dogs were made on skin locations for which dermatological examination was inconclusive. Subsequent pathological examination confirmed malignant melanoma in both cases. We suggest that these findings warrant a much more refined and multi-disciplinary study of the conditions under which detection of melanoma might be enhanced by biological or non-biological sampling of volatile chemicals emanating from skin.

REMOVAL OF THE VOMERONASAL ORGAN DOES NOT DISRUPT RECOGNITION OF MHC-DETERMINED INDIVIDUAL ODORTYPE

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In addition to its role in regulating immune function, the Major Histocompatibility Complex (MHC) of genes in mammals (H-2 in mice) also confers upon an individual a unique chemical signature or odortype. Several recent studies have implicated the vomeronasal organ (VNO) as critical in the recognition of individual odortypes while other studies have indicated that odortype information is detected by olfaction per se. We examined a possible role for the VNO in the recognition of MHC-odortypes in mice by first removing the organ (VNX) and then training the mice to distinguish, in a Y-maze, the odors of two congenic strains of mice that differed only in their MHC type. C57BL/6J mice (bb at H-2) and C57BL/6J-H-2^k(kk at H-2) provided urine for sensory testing. Eight VNX and six sham-operated, water-deprived (STRAIN) mice were trained to make the discrimination (mice were reinforced to choose the arm of the Y-maze that was scented with bb urine odors with a drop of water; the experimenter was blind to the surgical treatment). The average number of trials to reach and maintain criterion (> 80% correct choices) was 352 for VNX mice and 419 for the sham mice. Thus, the rate of learning this discrimination among VNX animals was normal relative to control animals and other non-operated animals trained for other studies. We conclude that the VNO is not involved in learning to discriminate between MHC odortypes.

ELECTROPHYSIOLOGICAL INVESTIGATION OF THE CARBON DIOXIDE SENSITIVITY IN THE BITING MIDGE

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Biting midges are minute biting flies in the family Ceratopogonidae. In this family, members of the genus *Culicoides* transmit a variety of human and livestock diseases. As with other biting flies, female *Culicoides* takes blood meals from vertebrates to obtain protein for oogenesis. It is during these repeated blood-feedings that diseases are transmitted from one host to another. In addition to transmitting diseases, *Culicoides* are also a significant nuisance insect, whose presence in large numbers adversely affects tourism and land development in many areas of the United States. Understanding what sensory signals the host releases, and how they are detected, may lead to strategies aimed at controlling this insect. It seems clear that olfactory cues play an important role in this behavior. In this study, we were interested in determining the adequate chemical stimuli for the sensilla located on the maxillary palps of female *Culicoides*. The basiconic sensilla of *Culicoides* share a general morphological similarity with sensilla found on the maxillary palps of mosquitoes, which contain neurons sensitive to carbon dioxide. Carbon dioxide is an attractant in other biting insects. We report here electrophysiological studies that have identified and characterized the neurons in these sensilla sensitive to low concentrations of carbon dioxide. We believe this type of research will lead to a better understanding of the sensory basis of host seeking behavior and to the development of strategies to control this insect pest. Portions of this work were supported by a SBIR grant #0033610884 to AJG.

A BEHAVIORAL PARADIGM FOR STUDYING OLFACTORY INDIVIDUAL RECOGNITION IN GOLDEN HAMSTERS

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In rodents, olfaction plays a key role in social recognition. Individual recognition may be crucial for many aspects of social behavior and it is also critical for the structure and stability of the relationships between individuals. In our lab, we took advantage of golden hamster aggressive behavior to develop a new behavioral paradigm. There are three crucial and unique characteristics of this paradigm. First, olfaction is highly required. Second, learning about individuals occurs rapidly. Third, the memory can be acquired in seconds but it lasts for a long time. In our experiments, two male golden hamsters interacted with each other and fought during 3 trials (with 3 min inter-trial interval). The loser was tested in Y maze after different periods of delay (30 min, 1 day, 3 days and 7 days). In Exp 1, the losers avoided approaching the arm with the winners that beat them in the open arena, whereas control males that did not fight did not avoid such winners. In Exp 2, the losers avoided familiar winners that beat them but did not avoid unfamiliar winners that beat other losers - rather, they approached the unfamiliar winners. In Exp 3, the losers were tested 7 days after the last interaction on the first testing day. Again, they kept away from the arm with the specific winners. These results showed that the losers learn to recognize individuals within few seconds and remember this information for both the short term (30 min) and the long term (at least one week). This behavioral paradigm is being used for further understanding of the neural basis of olfactory recognition and mechanisms of social memory.

WINNER AND LOSER EFFECTS ARE DEPENDENT UPON OLFACTION IN CRAYFISH (ORCONECTES RUSTICUS)

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Dominance hierarchies, the linear arrangement of individuals from dominant to submissive, are observed in many animals. It is believed that these hierarchies are reinforced through olfaction by receiving chemicals that express dominance. The urine odor appears to contain status cues that are believed to be paramount in the fight dynamics of crayfish. These chemical signals are detected by the antennules and can affect fight duration, and cause a winner–loser effect to develop. This effect occurs when a dominance hierarchy develops from a series of consecutive wins or losses in agonistic situations. The recent winner of a fight becomes more likely to win subsequent fights, whereas the loser becomes more likely to continue losing. The goal of this study was to determine if winner-loser effects were due to chemicals being released in the urine or if it was due to their "self-perception" of being a winner. The experiment allowed for normal agonistic interactions to occur as a control and then to allow interactions to occur with the fight opponent unable to detect chemical signals with their antennules. The blocked group was less likely to initiate a fight, compared to the control. The lesion study showed no difference in fight outcome, but the temporal mechanics of the fight greatly differed. When the crayfish were blocked from chemical senses they tended to quickly escalate in fight intensity. Olfaction may not play a key role in the outcome of a fight, but it seems control how these crayfish approach a fight.

105 Poster XIII. Recognition by Chemosensory Signals

KIN RECOGNITION BY ODORS IN GOLDEN HAMSTERS

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Kin recognition may influence many aspects of social behavior. We have developed behavioral tests to evaluate several aspects of the mechanisms underlying kin recognition. First, habituation experiments show that hamsters readily discriminate between the flank gland odors of siblings (either related to the subject or not related) if the subject has interacted with these individuals. Hamsters do not discriminate, however, between the odors of siblings if they do not know them, indicating that the odors of siblings are similar in quality. Second, hamsters show differential reactions to the odors of kin versus non-kin that fit with predictions based on preferential treatment of kin (less agonistic behavior, such as flank marking; more associations with kin) and mate choice (more sexual solicitation marking toward odors of non-kin). Third, hamsters show these reactions to unfamiliar kin and non-kin, indicating that they do not need to learn the identity of each of their relatives, but rather have a general mechanism for decisions about kin and non-kin (phenotype matching). Fourth, cross-fostering studies indicate that hamsters use a special type of phenotype matching that has been proposed by many but has never previously been unequivocally demonstrated, self-referent phenotype matching. That is, hamsters raised from shortly after birth in a foster family still, as adults, treat biological kin differently than non-kin. Together, these and other results indicate the subtlety and sophistication of olfactory perception and olfactory memory in the lives of animals.

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EVALUATION OF T2R1 AS A CANDIDATE GENE FOR THE PTC/PROP TASTE POLYMORPHISM

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Humans vary in how bitter they experience the taste of phenylthiocarbamide (PTC) and the chemically related compound 6-n-propylthiouracil (PROP). The ability to taste PTC and PROP is inherited, and recent linkage reports indicated that a gene responsible for this trait maps to human chromosome 5. A candidate gene within this region was recently proposed (T2R1): we sequenced this gene using genomic DNA from twenty-nine people with a range of phenotypes: 7 nontasters, 8 medium tasters, 12 supertasters and 2 subjects difficult to classify. Sequence variants were common in the upstream and protein-coding regions, but none were related to the ability to taste PROP. We conclude that T2R1 is unlikely to contain the PTC/PROP taste polymorphism and further work will be necessary to identify the gene or genes responsible for this trait. This work was supported by NRICGP/USDA 9603745 (VBD), NIH DC00283 (LMB) and NIH DC04698 (DRR).

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CLONING AND EXPRESSION OF ASIC1 FROM HUMAN FUNGIFORM PAPILLAE

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Acid sensing ion channels (ASICs) constitute a channel family that mediate the perception of acidotic pain in sensory neurons.To investigate the possibility that an ASIC functions as a sour taste receptor in human taste, RTPCR was performed with tissue obtained from fungiform papillae of human volunteers.Using the published sequence of human brain ASIC1,primers were designed to amplify its entire coding sequence. The PCR product from fungiform papillae was subcloned and sequenced. It differs from brain ASIC1 at aa 212, with Gly instead of Asp. Injection of the cRNA transcript of this clone into Xenopus oocytes, followed by two-electrode voltage clamp analysis, showed that altering the extracellular pH from 7.5 to 5.5 with HCl, acetic or citric acids yielded a rapidly-desensitizing inward current, whose magnitude was accurately correlated with the reported human psychophysical measurements of the relative sourness of these three acids. These data are consistent with the hypothesis that ASIC1 in human fungiform papillae participates in sour taste reception. RTPCR with papillae from 12 subjects showed variable expression of ASIC1,ASIC2a and ASIC2b, but no detectable expression of ASIC3. Support: NIH (F33DC00219, TH; DC03969, JGB); NSF (IBN 9816478, TH); DVA (JGB and SAM).

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UPTAKE OF AMPHIPATHIC TASTANTS BY TASTE CELLS

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Permeation of amphipathic tastants (i.e., those that possess hydrophilic and hydrophobic domains) into taste cells and their ability to activate G-proteins directly (*in vitro*), have led to the hypothesis that they may activate downstream transduction components directly, in addition to their action on apical G-protein coupled receptors. Quinine (Q), cyclo(Leu-Trp) (CLT), saccharin (SAC), and D-tryptophan (DT) permeated rapidly into (CV) taste bud cells against the concentration gradient and through the apical taste bud pore. Only Q and CLT do so in liposomes. SAC and DT permeated slowly into liposomes. DT accumulation in taste cells favored saturation whereas no clear saturation occurred for Q. The energy inhibitor carbonyl cyanide m-chlorophenylhydrazone (CCCP) inhibited DT uptake by CV taste cells. The results suggest that protein transporters may be involved in the uptake of DT and SAC, while uptake of Q and CLT may occur according to concentration gradient due to their binding to liposomes or cellular sites. Supported by grant I-2518-95 from BARD, The United States-Israel Binational Agricultural Research and Development Fund, and by a FAIR-CT-4040 travel grant from the European Commission.

IDENTIFICATION AND EXPRESSION OF CANDIDATE TASTE-RECEPTORS IN DROSOPHILA

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The ability to recognize and distinguish different chemosensory cues from the environment is essential for all animals' survival. Here we report the identification of a large family of putative chemoreceptor genes of Drosophila using a sequence search paradigm to identify hydrophobic proteins. The genes comprise a family of at least 50 genes and encode seven transmembrane receptors, with no apparent homology to any other known chemosensory receptors including known odorant and gustatory receptors of Drosophila, vertebrates and C. elegans . Using a transgene expression assay, we investigated the expression of eight of these genes and found that most of them are expressed exclusively in the gustatory system of the fly. As a rule, a given gene is expressed in only a few cells of any one of the four major gustatory sense organ of the adult which include the labellum, the cibarial sense organs, the taste sensilla on the legs and the taste sensilla on the anterior wing margin. One gene is expressed in cells of all gustatory sense organs and, surprisingly, also in a large number of cells of the olfactory system. Antibody staining of the cells expressing three distinct genes reveals bipolar cell morphology, indicating a neuronal identity of these cells. Moreover, visualization of the axons shows that these neurons project to the brain centers associated with chemosensory sensory information processing. Thus, we have identified a family of novel seven transmembrane receptor genes likely to encode contact chemosensory receptor proteins of the fruit fly.

A COMPARISON OF SODIUM CHLORIDE TASTE DETECTION IN C57BL/6J AND DBA/2J MICE

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Transduction of sodium is thought to occur via at least two mechanisms, one of which is amiloride sensitive (transcellular) and the other insensitive (paracellular). Amiloride, a sodium epithelial channel blocker, has been shown to reduce chorda tympani (CT) nerve responses and behaviorally-measured sensitivity to NaCl in rats. It also has been shown to suppress CT NaCl responses of C57BL/6J (B6) but not of DBA/2J (D2) mice. To test this strain difference behaviorally, we measured NaCl taste thresholds in these mice using a two-response operant discrimination procedure. B6 and D2 mice (n=8/strain), on a restricted water-access schedule, were trained, in a specially designed gustometer, to lick from one side spout in response to NaCl and from the opposite spout in response to water. Correct responses were reinforced with water. Because the amiloride-sensitive pathway is known to be necessary and sufficient for detection of weak NaCl solutions in rats, we predicted that B6 would have a lower threshold than D2. Unexpectedly, our results showed that the 2 strains had similar thresholds. This might mean that the paracellular pathway somehow compensates for the absence of the transcellular pathway, or that other gustatory nerves synapse on to amiloride-sensitive taste receptor cells and contribute to NaCl taste detection in D2 mice. To further distinguish between these possibilities, we are testing amiloride's effects on the NaCl taste sensitivity of D2 mice to see if it increases threshold as we have previously shown in B6 mice. Supported by NIDCD grants R01-DC01628 and R01-DC04574.

MOLECULAR ORIGINS OF THE SWEET TOOTH: A NOVEL TASTE RECEPTOR CONTROLLING THE AVIDITY FOR SUCROSE AND SACCHARIN IN MICE

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Differences in sweetener intake among inbred strains of mice are partially determined by allelic variation of the saccharin preference (*Sac*) locus. Genetic and physical mapping isolated a 194-kb interval containing the *Sac* gene. One gene within this interval (*Gpr98*) has a ~30% amino acid homology with several putative G protein-coupled taste receptors. *Gpr98* has two common haplotypes: one found in mouse strains with elevated sweetener preference and the other in strains relatively indifferent to sweeteners. Consistent with its role in taste, *Gpr98*is expressed in lingual tissue containing taste papillae. These data imply that *Gpr98* is a sweet taste receptor responsible for the phenotype of the *Sac* locus. This study is one of the first examples of the genetic dissection of a complex behavioral trait in mammals that culminated in the identification of a quantitative trait locus.

GPI ANCHORED PROTEINS IN THE CHEMORESPONSE OF PARAMECIUM TO FOLATE

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Some peripheral membrane proteins attach to the cell surface by glycophosphatidylinositol (GPI) lipid anchors. They are involved in signal transduction, and often found in aggregates of surface signaling molecules in special lipid domains (rafts). The receptor for folate uptake is GPI anchored and is a marker for anchors and rafts. We tested for the involvement of GPI anchored proteins in Paramecium chemoresponse, specifically for a GPI anchored receptor for the stimulus folate. Cells down-regulated for expression of PIG-A, which codes for an enzyme in GPI anchor synthesis, show few GPI anchored proteins, and display decreased response to folate and glutamate, but not to other stimuli. Antiserum against a mammalian folate binding protein (FBP) specifically blocks the folate behavioral response of normal cells, while other responses are unaffected. The same antiserum recognizes one 30kD protein among the GPI anchored proteins, and binds specifically on the surface of normal cells. A chicken anti-mouse FBP antiserum specifically immunoprecipitates a single protein of about 30kD, which is recognized by an antiserum against GPI anchors and binds to Clostridium alpha toxin, another test for the presence of a GPI anchor. We cloned two additional genes for another enzyme in GPI synthesis, PIG-K. They are being used for more antisense down- regulation of GPI anchoring. Other reagents in the study of GPI anchored proteins include Clostridium alpha toxin that requires GPI anchored protein receptors to kill cells. Sponsored by NIH and VCC.

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HIGH-THROUGHPUT ANALYSIS OF MRNA ABUNDANCE

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Virtually every phenotypic change or phenotypic difference, and many responses to stimuli, involve changes or differences in mRNA abundance. Simultaneously detecting multiple changes or differences obviously accelerates their discovery, but also reveals patterns of relationships among mRNA species. Because these relationships should stem from similarities and differences in the regulation of mRNA synthesis (transcription) and degradation, they often identify regulatory biochemical pathways. There are three main types of high-throughput analyses: (1) clone and count methods, (2) microarray methods, and (3) differential amplification/subtraction methods. Each has distinct advantages and disadvantages. These stem from differences in the number of transcripts tested, sensitivity, cost, speed, accuracy, and ease of use. Aided by the recent explosion of sequence information, these high-throughput methods are revolutionizing our ability to correlate gene expression with phenotypic change and stimulus response.

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DNA MICROARRAYS FOR NEURODEVELOPMENTAL GENE DISCOVERY—CLONE CHOICE, ANALYTICAL PATHWAYS AND DOWNSTREAM CONFIRMATION

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Advances in genetics and genomics have led to a number of new tools for the neuroscientist, the most widely used being microarrays. We have used focused, custom microarrays based on genetic subtractions to study gene expression in the developing and mature nervous system. To illustrate the power of this approach, our work with neural stem cells will be presented. We have performed a cDNA subtraction using representational difference analysis (RDA) in which neurosphere mRNA was subtracted from differentiated sister culture mRNA and vice a versa to discover, in an unbiased way, genes selectively expressed by CNS progenitors and not by their progeny. Genes enriched in stem cell cultures were screened by in situ hybridization. Several of the genes identified were selectively expressed in the developing neuroepithelium, consistent with their expression in neural stem cells. We have also used regular "unsubtracted" arrays containing 9k mouse genes and describe the overlap and non-overlap between the two approaches. About 1/2 of the genes identified using the subtractive approach were not on the 9K arrays. I discuss our microarray work in the context of methods for rapid downstream confirmation using alternative methods such as Northern blotting or in situ hybridization, as well as analytic approaches to array data analysis using standard statistical methods and bio-informatics.

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THE GENE EXPRESSION PROFILE OF THE AGING PROCESS AND ITS RETARDATION BY CALORIC RESTRICTION

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The gene expression profile of the aging process was analyzed in skeletal muscle of mice. Use of high-density oligonucleotide arrays representing over 11,000 genes revealed that aging resulted in a differential gene expression pattern indicative of a marked stress response and lower expression of metabolic and biosynthetic genes. Most alterations were either completely or partially prevented by caloric restriction, the only intervention known to retard aging in mammals. Transcriptional patterns of calorie-restricted animals suggest that caloric restriction retards the aging process by causing a metabolic shift toward increased protein turnover and decreased macromolecular damage. We have also examined the gene expression profile of the aging process in the ageing neocortex and cerebellum in mice. Ageing resulted in a gene-expression profile indicative of an inflammatory response, oxidative stress and reduced neurotrophic support in both brain regions. At the transcriptional level, brain aging in mice displays parallels with human neurodegenerative disorders. Caloric restriction selectively attenuated the age-associated induction of genes encoding inflammatory and stress responses. We are also using high-density oligonucleotide arrays and transgenic mouse models to determine the gene expression profile of the transcriptional response to oxidative stress, its alteration during the aging process, and its modification by caloric restriction. Our goal is to obtain hundreds of tissue-specific biomarkers of aging that can be used to evaluate genetic and pharmacological interventions.

116 Poster XVI. Cognitive and Mood Effects in Olfaction

EFFECTS OF OBSERVER NEGATIVE AFFECTIVITY ON PERCEIVED STRESS AND HEALTH SYMPTOMS TO AN AMBIGUOUS ODOR EXPOSURE

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Humans vary considerably in their reactions to odorous volatiles, some of which variation can stem from dispositional factors. In this study we investigated the role of Negative Affectivity (NA: a cluster of chronic negative emotions) using the Positive and Negative Affectivity Scale (PANAS). Subjects who scored high on NA were compared with subjects who scored low on NA before, during and after a 30-minute exposure to an ambiguous, balsam odor. Odor detection thresholds and the signal detection parameters of perceptual sensitivity and bias were evaluated pre- and post-exposure, while during exposure, subjects rated the intensity of odor, irritation, stress, anxiety and health symptoms they experienced. Salivary cortisol levels were also obtained at multiple timepoints during exposure as physiological indicators of psychological stress. Results showed no significant differences between groups for thresholds, sensitivity, or bias measures, but overall sensitivity scores decreased significantly after exposure ($p=.0007$). Consistent with predictions from our model, baseline levels of perceived stress and anxiety and some health symptoms were significantly higher in the high NA group, and post-exposure ratings for sensory irritation increased significantly from baseline ratings for the high NA group ($p=.026$). Although the propensity of high NA individuals to attend to and report somatic complaints from odors was supported by these results, the degree of reactivity may depend on both cognition and sensory properties of the odor. Supported by NIH DC 03704.

OLFACTORY FUNCTIONING AND COGNITIVE ABILITIES: A TWIN STUDY

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A Swedish version of the National Geographic Smell Survey (Wysocki & Gilbert, 1989) was completed by 227 twin pairs from the Swedish Adoption/Twin Study of Aging. Twins ranged in age from 45 to 89 years. Quantitative genetic analyses of four measures of olfactory functioning indicated moderate heritability for odor identification and perceived intensity, nonsignificant heritability for odor detection and perceived pleasantness. Bivariate analyses revealed that the relationship between odor identification and measures of verbal ability was primarily genetically mediated. The results provided further support for the hypothesis that odor identification and verbal ability in general tap the same cognitive domain (Larsson, 1997).

IS THERE PERCEPTUAL PRIMING IN OLFACTORY MEMORY?

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Several of the few studies that have investigated repetition priming in odor memory have concluded that olfactory judgments are possible to prime, that is, could be facilitated by previous exposure/processing. The generality of this observation, however, has not been impressive in comparison with observations for the visual modality. Moreover, it was suggested that the observed priming is of a conceptual type rather than perceptual type. A priming experiment designed to target perceptual repetition priming was therefore undertaken. Participants were first asked to judged the perceived similarity on a scale between a set of odors (primed set) and a comparison odor. Shortly after, participants were exposed to pairs of odors of either different or identical odors. Both types of odor pairs were sampled either from the primed set or from a new (control) set of odors. In response to the presentation of the first odor in the pair, reaction time (RT) for participants to indicate that they perceived the odor quality clearly was measured. The time of sensory integration measured this way was not faster for primed odors compared to controls. For the second member of the pair, participants judged whether the odor was identical to or different from the first member. Both RT and accuracy were measured here. Neither measure differentiated between primed and control odors. It was concluded that repetition priming does not seem to work at the level of sensory integration of odor quality. The idea of "perceptual priming" in olfaction as conceived in vision research was therefore rejected.

METACOGNITIVE ASPECTS ON ODOR KNOWLEDGE

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Three experiments investigated metacognitive aspects on odor knowledge. Experiment 1 took off from work of Cain et al. (1998) that showed that there was no reliable relation between feeling of knowing (FOK) judgments about odor identity and later memory performance. In contrast, our results indicated that FOK judgments about odor identity did predict actual knowledge reliably as assessed by a cued identity test, but with some overconfidence. Experiment 2 investigated retrospective confidence judgments (RCJ) in an odor-identification task and the so-called tip-of-the-nose (TON) phenomenon. RCJs were reliably correlated with correctness of identification, but the subjects were clearly overconfident in their answers. Results on TON experiences indicated that the subjects had none or extremely little partial information about the odor (name, source, etc.) as opposed to what is usually found in tip-of-the-tongue (TOT) studies. The resolution rate showed to be much weaker than in TOT experiments. Herz (1998, 2000) argued that the emotional impact of odor-evoked memories leads to the false impression that such memories are especially accurate, i.e. it leads to overconfidence. In Experiment 3 we investigated if the emotional intensity or valence of odors could explain the overconfidence for the RCJs observed in Experiment 2. No such relation could be observed. To conclude, metacognitive judgments in olfaction are positively related to actual knowledge of odors, but overestimate the amount of it for unknown reasons.

PSYCHOLOGICAL EFFECTS OF 250μM ANDROSTADIENONE: MOOD, STATE AND TRAIT ANXIETY

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Exposure of women to the putative pheromone androstadienone has in several experiments been shown to induce changes in measures of mood and ANS activity in the absence of a perceptual awareness of the substance's presence. In the current experiment we replicated one of these experiments (Jacob & McClintock, 2000). 250μM androstadienone (A) was dissolved in propylene glycol (PG) and masked with eugenol (E), which supposedly prevented participants from discriminating between the test (A+PG+E) and the control (PG+E) stimuli. Forty women participated on day 13 or 14 of their menstrual cycles in two seemingly identical experimental sessions, one involving exposure to the test stimulus and the other to the control stimulus. In each session, the participants were asked to fill in two inventories (STAI-S and STAI-T) providing indices of state and trait anxiety, and eight VAS scales measuring different moods. The stimulus was then applied to the upper lip of the participant after which she spent approx. 20 minutes browsing through travel magazines. Anxiety and mood were then measured again. Finally, the participants LH-levels were measured. ANOVAs of the ten dependent measures indicated that the pre/post-exposure change in the feeling of being focused was the only variable that was (positively) affected by exposure to androstadienone ($p=.003$). As opposed to what has been indicated in previous research, the test and control stimuli were found to be discriminable ($p=.0004$). However, discriminatory performance as well as LH-levels did not affect the reactivity to androstadienone.

ODOURS IDENTIFICATION AND DISCRIMINATION : INFLUENCE OF CULTURE AND TYPICALITY ON PERFORMANCE

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Cultural effects may positively or negatively influence the identification of odours. We tried to evaluate the effect of different European cultures on two tasks : odour identification and odour discrimination. We used three olfactory tests designed in three European countries : Sniffin'sticks (Germany), Scandinavian Odour Identification Test (Sweden) and Olfactory Clinical test (France). The tests were administrated to 80 healthy elderly subjects (55-79 years old) in three European countries (Netherlands, Sweden and France). Our results showed differences in scores of odour identification between the three countries. More surprisingly, they evidenced a cultural effect on the discrimination task between France and Sweden. The influence of culture on identification and discrimination varies across odours. Thus, for some odours there is a cultural effect on identification and also discrimination. Cultural differences were also evidenced by typicality evaluations in the three countries. This study is aimed at developing more "transcultural" olfactory tests and the data presented here are very useful to reach this goal.

ODOR IMAGERY AND DETECTION OF PERI-THRESHOLD ODORS

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The existence of mental imagery for odors has been difficult to prove objectively. Mental imagery has been shown to influence perception in the visual and auditory modalities, and particularly so through detection of weak signals. We used the same approach for olfaction. The purpose of this study was to provide further evidence that people are able to create mental images of odors, and to explore gender differences in this ability. We tested 24 healthy subjects (12 women). We first determined subjects' detection thresholds for Phenyl Ethyl Alcohol (PEA) and Citral. Subjects were then given 100 detection trials: an odorant at the threshold level (either PEA or Citral) paired with a blank was presented, and subjects indicated which one smelled stronger. Simultaneously with these tasks, subjects were asked to imagine the odor of either a rose (PEA) or lemon (Citral). Thus each subject was given 50 detection trials in which the presented and imagined stimuli were the same (matched imagery) and 50 trials in which the two stimuli were different (mismatched imagery). The difference between matched and mismatched imagery was significant: subjects were better in detecting peri-threshold olfactory stimuli when they were simultaneously imagining that odor than when they were simultaneously imagining an odor other than the one being presented for detection. There was a tendency for this difference to be larger in women. We interpret our findings as providing objective evidence for the existence of olfactory imagery. The possibility that women may be better than men in imagining odors awaits further confirmation.

EPISODIC ODOR RECOGNITION – THE INFLUENCE OF SUBJECTIVE ODOR EXPERIENCE

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The purpose of the present study was to further our understanding of the influence of subjective experience of odors on episodic odor memory. Ninety-eight men and 104 women (19-91 years of age), rated their experiences of a number of odors on various dimensions (i.e., pleasantness, intensity, irritability). Subjects were then assessed in an episodic odor memory task including the previously rated odors. Preliminary results suggest that pleasantness is related to episodic odor memory performance such that the more unpleasant the odors are perceived the more likely it is that they will be remembered across adulthood. A pleasantness by sex interaction was also obtained. The source of this interaction was that men remembered unpleasant and pleasant odors equally well, whereas women performed at a particularly high level for unpleasant odors. Additional data indicate that the more intense the odors are experienced the more likely they are to be remembered. Of further interest was the negligible effect of irritability, tapping activation of the trigeminal nerve, on episodic odor memory performance. Supported by a grant from The Swedish Council for Research in the Humanities and the Social Sciences.

THE EFFECT OF TASK REQUIREMENTS ON P300 HABITUATION IN THE OLFACTORY EVENT-RELATED POTENTIAL (OERP)

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This study focuses on habituation of the P300 in the OERP. Previous research has examined P300 habituation and its relationship to task requirements. It suggests that stimuli requiring an increase in allocation of attentional resources slow the effects of habituation. The goal of this study was to compare variance in demands on attention by three different paradigms and their effects on reducing habituation . The olfactory stimulus, amyl acetate, was presented by an olfactometer which has been previously described (Murphy, et al., Chem. Senses, 19, 1994). A target stimulus, pyridine, was used for the odd-ball paradigm. OERPs were recorded monopolarly at the Fz, Cz, and Pz electrode sites. Each recording session consisted of 20 trials with an inter-stimulus interval of 60s. Stimulus paradigms were presented in a counter-balanced order with an inter-block interval of 20 minutes. Brain potentials were amplified and digitized, and a grand average was created for each stimulus condition. Component amplitudes were selected by choosing the largest positive or negative peak during corresponding latency windows (N1= 300-500, P2= 450-700, N2= 600-900, P300= 750-1100ms). Repeated measures ANOVAs demonstrated no significant differences among paradigms in P300 amplitude or latency. These results indicate that the three paradigms are comparable to one another in their effects on habituation in the OERP. Supported by NIH grant # DC02064 to CM.

125 Poster XVI. Cognitive and Mood Effects in Olfaction

INFLUENCE OF ODORANT EXPOSURE ON REMEMBERED ODORANT QUALITY

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Odor memory research suggests that, once established, the mental image of an odorant is relatively stable, either from the passage of time or the learning of new material. We examined the degree to which odor memories could be modified by exposure to actual odorants and whether these changes were incorporated into more permanent odor memory stores. Subjects rated pair-wise dissimilarity between their memory representations of each of six odors (Cinnamon, Mint, Orange, Rose, Rubbing Alcohol, and Vanilla) in a Memory Task and also rated pair-wise dissimilarities for odorants representing each of these qualities (Cinnamaldehyde, R-Carvone, d-Limonene, Phenethyl alcohol, Propanol, Vanillin) in an Odorant Task. Subjects first completed the Memory Task, then the Odorant Task and a second Memory Task. A third memory task was completed 1 hour later. Rated odor dissimilarity fell from an average of 82.9 in the Memory Task to 66.8 in the Odorant Task ($p<0.0002$, Paired t-test). This relative perceptual similarity between the odorants in the Odorant Task suppressed odorant dissimilarity stored in memory to an average of 75.6 (Memory Task #1 vs #2, $p=0.05$). The suppression remained an hour later but odorant dissimilarity in Memory Task #3 (77.1) was returning to the levels seen in the first Memory Task (Memory Taste #1 vs. #3, $p=0.07$). Therefore, memory stores of odorant quality appear to be mutable in the short term but return to pre-exposure levels in the long term.

126 Poster XVI. Cognitive and Mood Effects in Olfaction

HUMAN OLFACTORY DISCRIMINATION BETWEEN NEUTRAL AND EMOTIONAL STATES

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Olfactory communication of emotions, particularly of fear, is widely reported in animals. In a previous study, we suggested that this may be the case in humans too. Women identified above chance odors of people who were happy and odors of men who were afraid. The purpose of this study was to extend our previous work by determining (1) whether people discriminate between odors collected from the same person during neutral and an emotional state occurring within minutes of one another, (2) whether olfactory evaluations of one's own emotional state are more accurate than those made by other people, and (3) whether evaluations based on odors of individual donors and of odors combined by gender yield comparable results. We collected underarm odors on gauze pads from 6 healthy non-smoking young adult men and women on three occasions while they watched a neutral video followed by an emotional video. The videos had been tested previously to respectively produce the emotions of neutrality, fear, sexual arousal, and happiness. Odors from individual donors and odors combined by gender were separately evaluated on paired comparison, ranking, and identification tasks. We found that overall: (1) people discriminate between a neutral and an emotional state based on odors collected within minutes of one another, (2) self evaluations are more accurate than those by others, and (3) olfactory evaluations of individual donors and of odors grouped by gender yield somewhat different results. Together, the findings extend our previous findings, and have important implications for understanding human emotional communication.

127 Poster XVI. Cognitive and Mood Effects in Olfaction

THE EFFECTS OF HEMISPHERIC LATERALISATION OF ODOUR PROCESSING ON TASK PERFORMANCE AND MOOD

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Hemispheric lateralisation in odour perception and naming has been indicated by the work of a number of researchers. Two studies were conducted by the authors to further examine the effect of this phenomenon on task performance and mood assessment. In one experiment, subjects were completed a series of tasks while an odour was presented to the left, right, or both nostrils, depending on the condition to which they were assigned. Each of these tasks was associated more strongly with processing in the left or the right hemisphere. It is expected that some degree of hemispheric priming will occur when an odour is presented to a single nostril and this will result in significantly different levels of performance on those tasks associated more strongly with the hemisphere that is primed. The second study involved assessment of mood pictures. Subjects were shown a series of mood pictures which were previously rated neutral, while an odour was presented to either the left, right, or both nostrils depending on the subject's assigned condition. It is expected that ratings for the neutral pictures will be higher for subjects presented with odour to their right nostril, relative to ratings in the left- or both-nostril conditions.

128 Poster XVII. Modulation of Chemosensory Systems

CAPSAICIN MODULATES VOLTAGE-GATED NA, K, AND CA CHANNELS

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Application of capsaicin to nociceptors containing vanilloid receptors induces the generation of action potentials. However, upon repeated capsaicin applications these nociceptors become refractory to many stimuli including capsaicin. Although much is known how capsaicin excites neurons, little is known about how it inhibits them. We investigated downstream pathways from capsaicin/receptor ion channels in capsaicin-sensitive (CS) and -insensitive (CIS) TG neurons that modulate voltage-gated sodium (TTX-s), (TTX-r), potassium (IA) and calcium channels (ICa). Using EIA and essays for PKC activation we found that capsaicin activates cAMP ($K1/2 = 0.16$ uM), cGMP ($K1/2 = 0.12$uM) and PKC, and that their activation is inhibited by capsazepine (CPZ). Voltage-clamp studies found that capsaicin receptor activation activates the above second messenger pathways that inhibits VGSCs (0.6uM), IA (1.4uM) and ICa (0.24uM) in CS neurons. Incubation with CPZ will prevent capsaicin's inhibition. CIS neurons were only inhibited about 25% at very high capsaicin concentrations (> 10uM). Investigations of CS neurons revealed that for cAMP the peak TTX-r VGSCs, IA, ICa currents, decreased (+8%), did not change (1%) and increased (10%), respectively. For cGMP, the changes were,-20%, +30%, + 12%, respectively. For the PKC activator, PDBu, the changes were +20%, 0%, 0%, respectively. This suggests that in CS neurons capsaicin modulates different voltage-gated ion channels in a manner that is dependent on particular intracellular mediator. The selective modulation of voltage-gated ion channels by the activation of vanilloid receptors may explain many of its diverse physiological effects on taste as well as other sensory systems.

EPHAPTIC INTERACTIONS IN THE MAMMALIAN OLFACTORY SYSTEM

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Olfactory discrimination begins with the binding of odorants to olfactory receptors expressed by neurons in the nasal epithelium, each neuron expressing a small number (1-2) of receptors. This information is relayed to the main olfactory bulb through an unmyelinated, un-branched axon. Axons originating from different neurons coalesce into densely packed fascicles, suggesting that neighboring axons may influence each other via current spread through the extracellular space, *i.e., via* ephaptic interactions. We tested this hypothesis with the use of computational and experimental approaches. Numerical solutions of models of a fascicle show that significant ephaptic interactions occur for a range of physiologically relevant parameters. An action potential in a single axon in a fascicle can evoke action potentials in all other axons in that fascicle. Ephaptic interactions can also lead to synchronized firing of independently stimulated axons. Functional imaging of voltage-sensitive dye signals in an *in vitro* slice preparation of rat olfactory axons supports the existence of significant inter-axonal interactions. These findings suggest that ephaptic interactions among neighboring axons are important determinants of the nature of the olfactory discrimination code.

DIRECT EVIDENCE FOR THE PRESENCE OF A CA2+ DEPENDENT K+ CHANNEL IN OLFACTORY CILIA

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We have previously documented that toad olfactory receptor neurons (ORNs) can be inhibited by odorants, and that this process involves a Ca2+-dependent K+ channel. An odorant-induced K+ current with similar properties was found in rat, in which ORNs can also be inhibited by odorants. Indirect evidence suggested the presence of this channel in the chemosensory cilia, but definitive proof was missing. We addressed this problem by investigating whether a Ca2+-dependent K+ (KCa) channel could be recorded from inside-out membrane patches excised from chemosensory cilia. Using high-resistance pipettes, we were able to record single ciliary KCa currents. The channel was highly dependent on micromolar Ca2+ levels on its cytoplasmic side. We looked for independent evidence for the presence of KCa in the cilia. Since the inhibitory K+ current is sensitive to iberiotoxin, a specific blocker of the big conductance KCa, we tested whether an antibody against this channel could detect a similar channel in the cilia. The antibody revealed a 180 KD protein in Western blots of a purified ciliary preparation. This fraction also exhibited type III adenylyl cyclase, which was absent in the deciliated olfactory epithelial fraction. Our results demonstrate that olfactory cilia contain a Ca2+ dependent K+ channel, which is likely to be the channel responsible for the inhibitory odor response. Supported by Fondecyt 1990938, Presidential Chair in Science, ICM P99-031-F (JB) and Fondecyt 1000824 (JS).

PONTINE GUSTATORY PROCESSING: MODULATION BY DIFFERENT SOURCES OF CENTRIFUGAL INPUT

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Recently we characterized how electrical stimulation of the central nucleus of the amygdala (CeA) modulates baseline and taste-evoked activity in the parabrachial nucleus (PBN) (Lundy and Norgren, J. Neurophysiol. 2001). The present study recorded extracellular responses of PBN neurons to lingual application of sucrose, NaCl, citric acid, and QHCl before, during, and after electrical stimulation of the gustatory cortex (GC), the lateral hypothalamus (LH), and the CeA. To date, the sample is small — 8 NaCl-best and 2 acid-best neurons have been tested during stimulation from all 3 forebrain sites. In a single NaCl-best cell, electrical stimulation in all 3 areas inhibited spontaneous activity and responses to 2 tastes. Three others were inhibited by GC and CeA; another by GC alone. Cortical stimulation inhibited spontaneous discharge in another cell without affecting taste-evoked activity, while the remaining 2 NaCl-best neurons were unaffected by forebrain stimulation. In 1 of the 2 acid-best cells, stimulation of the GC and the CeA inhibited spontaneous activity, but not taste responses. LH and GC activation inhibited both spontaneous discharge and taste responses in the other acid-best cell. These preliminary results demonstrate that descending input from the GC, the LH, and the CeA alter gustatory processing in the dorsal pons and that some PBN taste neurons receive centrifugal input from more than one forebrain area. Supported by NIH DC00369, DC00240, MH43787, and MH00653.

NEURAL ACTIVITY IS MODULATED BY METABOTROPIC GLUTAMATE RECEPTORS IN RAT OLFACTORY BULB SLICES

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In the main olfactory bulb (MOB) mitral/tufted cells express high levels of Group I metabotropic glutamate receptors (mGluR1) whereas granule cells express both Group I (mGluR5) and Group II mGluRs (mGluR2). Here, we characterized the effects of mGluR agonist/antagonists on mitral and granule cells in MOB slices using whole-cell patch clamp recording. In mitral cells, bath application of trans-ACPD, an agonist for group I/II mGluRs, initially resulted in strong membrane potential depolarization with increased action potential firing rate followed by spike broadening, decrease of spike amplitude and gradual reduction in firing (i.e., depolarization block). These effects persisted in mitral cells that were pharmacologically isolated from fast synaptic transmission indicating that ACPD directly excites mitral cells. MCPG, a non-selective group I/II mGluR antagonist blocked partially to fully the excitatory response of mitral cells to ACPD. ACPD depolarized granule cells transiently with strong firing of action potentials. With blockers of fast synaptic transmission in the bath, ACPD resulted in smaller depolarization and did not cause increased spiking in granule cells. In blockers (APV, CNQX), under voltage clamp, ACPD increased the frequency of IPSCs in mitral cells. This suggests that the excitatory effect of ACPD on granule cells is due to both an indirect effect mediated by mGluRs on mitral cells which in turn activate granule cells as well as a direct effect that remains in the presence of blockers. Support PHS grants: DC03195, DC02588, DC00347 & NS36940.

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) MODULATES ELECTRICAL PROPERTIES OF OLFACTORY BULB NEURONS

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Neurotrophins support a rapid neuromodulatory role in addition to their classical functions in growth and differentiation. The potassium channel Kv1.3, predominantly found in the olfactory bulb (OB), was acutely (15 minutes) modulated by BDNF to cause current suppression but not changes in $V_{1/2}$, inactivation kinetics, and deactivation kinetics. Such modulation was not observed upon stimulation with NGF or NT3, even though Western blot analysis demonstrated expression of all three neurotrophin receptors, namely, TrkA, TrkB, and TrkC in OB membrane preparations. TrkB-specific tyrosine kinase inhibitor (TKI) K252a, but not the inactive structural analog K252b, blocked BDNF-induced current suppression of Kv1.3 in cultured OB neurons. Tyrosine phosphorylation of Kv1.3 increased two fold when OBs were acutely stimulated with BDNF. This increased phosphorylation was blocked by preincubation with TKIs (K252a, Lavendustin A, Herbimycin A). When OB neurons were chronically stimulated (24-216 hrs) with neurotrophins NGF, BDNF, or NT3, we found incremental increases in peak current amplitude and speeding of the inactivation and deactivation kinetics for neurons treated with BDNF. Lastly, sensory deprivation, as induced by unilateral naris-occlusion, caused an increase in BDNF-induced tyrosine phosphorylation of Kv1.3 that was not due to increased expression of TrkB but rather an increased efficacy of the kinase. We are using site-directed mutagenesis of key phosphorylation motifs in the channel to further study BDNF pathways in the OB. This work was supported by NIH R29DC03387.

PHYSIOLOGICAL ACTIONS OF CHOLECYSTOKININ ON RAT TASTE RECEPTOR CELLS

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Our laboratory has previously localized the neuropeptide cholecystokinin (CCK) to a subset of taste receptor cells in rat lingual tissue and additionally demonstrated that taste cells respond to exogenously applied CCK with elevations of intracellular calcium. In this study, we show that ion currents are modulated by CCK and begin pharmacological analysis of the receptor subtype and transduction mechanism underlying these events. Using perforated patch clamp techniques on dissociated cells, exogenous application of CCK (0.1 and 10 μM) caused an inhibition (♠20%) of an outward potassium current in most cells though a strong enhancement (up to 50%) of this current was observed in a minority of cells. These effects were long lasting (up to 60 minutes), consistent with many modulatory processes. Inhibitions or enhancements could be blocked by the non-specific CCK receptor blocker proglumide (50 μM) and or the CCK-A specific blocker lorglumide (0.1 μM). H89 (50 nM), a protein kinase A inhibitor, was unsuccessful in blocking the CCK-induced inhibition whereas bisindoylmaleimide (100 nM), a protein kinase C inhibitor, prevented the CCK-induced inhibition, suggesting involvement of the inositol trisphosphate (IP_3) system. Using ratiometric imaging techniques, CCK induced elevations of intracellular calcium could also be inhibited by proglumide and lorglumide. Taken together, these observations suggest that CCK may stimulate taste cells via CCK-A receptors elevating intracellular calcium via IP_3 production with consequent downstream results on potassium currents. Supported by NIH DC-00401 and NSF IBN-9724062.

TASTE-RESPONSIVE NEURONS IN THE HAMSTER SOLITARY NUCLEUS ARE MODULATED BY THE LATERAL HYPOTHALAMUS

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Taste-responsive cells of the nucleus of the solitary tract (NST) can be modulated by descending influences, which could play a role in the changes in taste activity after alterations in blood insulin or glucose or following taste-aversion learning. The lateral hypothalamus (LH) is a regulatory center for feeding behavior and metabolic homeostasis. We determined the percentage of taste-responsive cells that are under the influence of the LH in urethane-anesthetized hamsters. Bipolar electrodes were positioned bilaterally in the LH. Taste-responsive neurons were tested with sucrose, NaCl, quinine hydrochloride and citric acid. After establishing taste profiles for each cell, rectangular pulses (0.5 ms, 0.1 mA, 1/3 Hz) were delivered to the LH. Among 106 taste-responsive cells in the NST, 46 responded to LH stimulation (43.4%). Action potentials were evoked in 18 cells, whereas the spontaneous activity was inhibited in 11 cells by ipsilateral LH stimulation. Contralateral LH stimulation elicited excitatory responses in 33 cells and inhibitory responses in 3. In 13 cells, the spontaneous activity was modulated bilaterally. Injection of DL-homocysteic acid into the LH in a subset of these cells mimicked the effects of electrical stimulation. In 6 cells tested simultaneously with both electrical stimulation and taste stimuli, electrical stimulation modulated the taste-evoked responses. These results suggest that the processing of gustatory information in the NST can be modulated by activity within the LH. Supported by NIDCD Grant DC00066 to D.V.S.

INFLUENCE OF THE AMYGDALA ON TASTE NEURONS IN THE SOLITARY NUCLEUS OF THE HAMSTER

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Neuroanatomical studies have shown that the nucleus of the solitary tract (NST) receives descending inputs from several areas of the gustatory forebrain, including the central nucleus of the amygdala (Ce). We examined the influence of Ce stimulation on the activity of NST taste cells. Concentric bipolar stimulating electrodes were stereotaxically implanted into the Ce bilaterally. Taste solutions were sucrose, NaCl, quinine hydrochloride, and citric acid. When a taste-responsive cell was isolated in the NST, its taste response profile was determined and then rectangular pulses (0.5 ms, 0.1 mA) were delivered to the Ce at 1/3 Hz. Stimulation of the Ce orthodromically activated 32 of 129 taste-responsive NST cells (24.8%). Seven of these cells were inhibited and fourteen were excited by ipsilateral Ce stimulation. In 29 of these 32 cells, stimulation of the contralateral Ce produced excitatory responses in 21 and inhibitory responses in 5 cells. Eight NST cells responded to bilateral Ce stimulation. DL-homocysteic acid microinjected into the Ce mimicked electrical stimulation in 7 cells that were tested with this protocol. Six cells were tested for the effects of electrical stimulation of the Ce on the responses to taste stimulation, which were enhanced in 6 cells and reduced in one other. These results demonstrate that descending projections from the Ce, in addition to those previously shown from gustatory neocortex and concurrently from the lateral hypothalamus, influence the activity of taste-responsive cells in the NST. Supported by NIDCD Grant DC00066 to D.V.S.

ZINC HAS EXTRACELLULAR AND INTRACELLULAR EFFECTS ON RAT OLFACTORY BULB GLYCINE RECEPTORS

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Zinc (Zn) is released by neuronal depolarization and affects the function of various neurotransmitter receptors. We and others have shown that low micromolar concentrations of Zn, coapplied extracellularly with glycine (G), potentiate currents mediated by low micromolar concentrations of G; higher concentrations of Zn block them. Yet, coapplied extracellular Zn has little effect on currents mediated by high concentrations of G, suggesting that saturation of G binding sites prevents its effects. The present experiments suggest that Zn can inhibit currents mediated by high-micromolar G if *preapplied* extracellularly but potentiates G currents when applied intracellularly. Under whole-cell voltage-clamp conditions, cultured neurons were perfused extracellularly with Zn for 15 sec to 5 min, then perfused with G. Zn significantly reduced G currents in over 95 percent of examined neurons, with increasing effect with longer Zn exposure (up to 2-3 minutes). Despite the increased effect of Zn with long exposure, intracellular application of zinc chelators did not reduce it. This suggests that an extracellular site is mediating zinc's inhibition of G currents. In contrast, Zn preapplied intracellularly potentiated currents induced by high-micromolar G by 40-80 percent. These, and previous results, suggest that Zn can affect G channel function by both extracellular and intracellular mechanisms. Supported in part by NIH/NIDCD.

METHIMAZOLE ALTERS GLUTATHIONE DEPENDENT-ENZYME EXPRESSION AND ACTIVITY IN THE MOUSE OLFACTORY MUCOSA

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Mackay-Sim and Beard (Dev. Brain Res. 36:181, 1987) demonstrated that prolonged treatment of mice with the anti-thyroid drug propythiouracil (PTU) causes olfactory dysfunction. Methimazole (MMI) is another anti-thyroid drug known to negatively affect the olfactory system of rodents. We have demonstrated that PTU treatment significantly alters glutathione-dependent enzymes in the mouse olfactory mucosa. In this study, we investigated the effect of 1 and 2 months oral administration of MMI on olfactory GST alpha and mu isozyme expression, and total olfactory GST activity in the mouse. We also evaluated the impact of MMI treatment on olfactory glutathione peroxidase (GSH-PX) activity. MMI treatment significantly induced GST alpha and mu expression at 1 month. GST alpha and mu expression was slightly, but not significantly elevated at 2 months of treatment. Total GST enzyme activity did not change after 1 month of MMI treatment. In contrast, treatment at 2 months caused a notable decrease in GST activity (53%). GSH-PX activity decreased significantly after 1 and 2 months of MMI treatment(36% and 44% respectively). These data suggest that MMI may induce oxidative stress conditions which contribute to olfactory dysfunction. The mechanisms responsible for altered GST expression remain to be elucidated. Supported by HUMAA Endowed Founders Chair Award to E.W. and the Howard University Specialized Neuroscience Research Program (NIH/NINDS 1 U54NS394007).

THE QUALITY CLASSIFICATION OF ODORS IN A 3D OLFACTORY SPACE

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The classification of odors according to their quality discrimination is an old challenge, often solved by using rules that are more empirical than experimental. The here presented 3D olfactory space for 141 odorants explains 88% of the experimental psychophysical variance. It also displays 16 major semantic descriptors, represented by 16 correspondent colors. Each color appears as reasonably well localized into the space, suggesting a continuum rather than a mosaic of clusters. Technical precision. Dravnieks and the ASTM developed the systematic characterization of odorants by a limited number of semantic descriptors (146). However, factor analysis of the data obtained with this method gives rise to a rather cumbersome 17-dimensional space explaining 89% of the variance, difficult to handle. On the other hand, multidimensional scaling (MDS) applied to direct measurements of similarities yields a 3D-space explaining 86% of the variance, but for a limited number of odorants. Practically, the geometrical growing of experiments for this type of procedure prevents to apply it to more than 25 odorants. The advantages of both procedures to characterize the olfactory quality can be combined, by using an algorithm which from semantic profiles, leads to similarities comparable to that obtained directly by human subjects. The final space results from the MDS applied to the similarity data derived from the semantic profiles of Dravnieks for 141 odorants.

A HIGH-THROUGHPUT METHOD FOR ASSESSING TASTE FUNCTION IN INDIVIDUAL MICE

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The ability of chemosensory biologists to identify genetically-based alterations in murine taste function has been impeded by the absence of high-throughput screening procedures. To fill this void, we have been developing brief-access testing protocols for assessing taste function in laboratory mice. We provide mice with access to a single taste stimulus during 5-sec trials, and then measure their licking responses with an automated gustometer. During a single 30-min session, we can test several taste stimuli in a randomized order, and derive concentration-response functions from individual mice. Because each trial is brief, we can be relatively confident that the licking responses are under orosensory control and not confounded by postingestive effects. We have customized a method for deriving robust measures of taste responsiveness to QHCl, NaCl, and sucrose in the C57BL/6J mouse. This method screens wildtype or mutagenized mice for oromotor function and taste responsiveness in as little as 10 days. Individual mice are run through the screening protocol and then their behavior is referenced to a normative database based on the responses of 100 C57BL/6j mice. Any mouse that qualifies as a statistical outlier can be flagged for further testing. We intend to make this high-throughput screen freely available to the scientific community, and sufficiently automated so that it could be performed by a technician with limited experience. This project was supported by R01-DC04475.

A PAPER TEST FOR PROP TASTER CLASSIFICATION THAT MINIMIZES EXPOSURE TO PROP

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Taste sensitivity to the bitter thiourea compound 6-n-propylthiouracil (PROP) is genetically determined and may be a marker for individual differences in oral sensory perceptions, food acceptance and body weight. Access to brief, reliable methods for PROP taster classification would be a valuable tool in large-scale studies that seek to relate PROP sensitivity to nutrition and health. Tepper et al.(*Chem. Senses* 75: 616, 2000)described methods in which subjects rated the intensity of solutions of PROP and NaCl in 3-solution or 1-solution tests. Here we describe a paper test based on this earlier work. Filter paper disks were soaked in 50 mM PROP or 1.0 M NaCl and oven dried. 125 adults participated in the study. Group 1 (n=63) rated the intensity of the disks placed on the tongue using the Labeled Magnitude Scale. They were classified as nontasters (n=18) medium tasters (n=30) or supertasters (n=15) using numerical cutoffs obtained by constructing 95% confidence intervals around the group means for PROP. The cutoff score for nontasters and supertasters was ≤15 and 67, respectively. Group 2 (n=62) was classified using the cutoffs obtained for Group 1. Subjects were previously classified using the 3-solution method. The co-efficient of association between the 3-solution and the paper test was 0.76 for Group 1 and 0.73 for Group 2, indicating high agreement between methods. These data suggest that the paper disk method is a valid and reliable test for taster classification that has the added advantage of minimizing exposure to PROP (187 μg PROP/disk).

MODELING THE TIME COURSE OF BURN PRODUCED BY CAPSAICIN, PIPERINE, ZINGERONE, AND MENTHOL

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Previous experiments in this series showed that psychophysical responses to capsaicin can be modeled as the sum of three processes: a phasic (or "change" detection) mechanism, a tonic (or "level" detection) mechanism, and a rising (or cumulative irritation) function that may be characteristic of painful stimulation. The model permits us to account for single and double step-increases and for step decreases in concentration and for individual differences by changes in the gains of the three processes. In this study, subjects were given continuous, 34-minute stimulation with either capsaicin, piperine, zingerone, or menthol presented on filter papers. The model fit the responses to these irritants with the same time constants obtained for each process in our earlier studies using capsaicin. Differences among irritants were accounted for by differences in the gains of the three processes. The time course of the response to menthol was similar to capsaicin, having a large phasic gain and moderate integrator gain. Zingerone showed a large phasic gain only, indicating considerable "adaptation." Finally, piperine yielded moderate tonic and phasic gains. The patterns of gains for each compound allow us to predict patterns of interaction (cross adaptation) between these irritants.

FUNCTIONAL MEASUREMENT OF CATEGORY AND LABELED DISSIMILARITY SCALES IN JUDGMENTS OF SWEETNESS

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This study used judgments of sweetness differences to examine the validity of category and labeled dissimilarity scales. The category scale had difference categories spaced according to equal intervals. The labeled dissimilarity scale had difference categories spaced according to magnitude estimates of their perceived relative intensities (Kurtz, White, & Hayes, 2000). Ss were assigned randomly to one of the scaling conditions. The task was to rate the difference in sweetness intensity elicited by pairs of sucrose solutions of different molar concentrations in a 5 (0, .1, .2, .4, and .8) by 3 (0, .2, and .8) design. Functional measurement was used to test if the difference ratings fit a subtractive model (i.e., parallel simple effects), which would suggest that the difference ratings were linear, unbiased estimates of differences between the actual sensory magnitudes (e.g., Anderson, 1981). Results demonstrated some deviations from a subtractive model for category and labeled dissimilarity scales. These results have implications for an ongoing debate in psychophysics regarding whether category scaling or magnitude estimation is valid given that their results do not always agree.

THE CEPHALIC PHASE SALIVARY RESPONSE: ROLES AND RELATIONS

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The cephalic phase salivary response (CPSR), has been proposed as an objective "diagnostic index" for an array of personal states (e.g., hunger) and characteristics (e.g., extroversion). A series of studies explored new clinical applications and environmental factors that may confound them. First, a positive association was hypothesized between the CPSR and oral health measured by the decayed, missing & filled (DMF) index. Saliva was collected for 3 minute periods while viewing either a pen or orange juice in 20 adults whose dentists independently completed a DMF form. A significant association ($r = 0.46$, $p<0.05$) was observed. The CPSR predictive power for adverse foods reactions was tested in subjects viewing orange juice and a pen who believed it was wholesome (N=25) or likely to cause illness (N=83). Subject with symptoms had higher CPSR ($p<0.05$). Based on 10% greater response to the juice, the CPSR had a sensitivity of 57% and specificity of 92%. The influence of culture and dietary experience on the CPSR was tested by comparing responses in Americans and Ghanians to peanuts, peanut butter, almonds, chocolate, chestnuts, pickles and rice cakes. The pattern of CPSR to the various foods differed markedly between populations. In neither group was the CPSR clearly related to hypothesized relevant properties of the foods (e.g., fiber, acidity), but did reflect differences in food familiarity. These studies have identified new functional and potentially diagnostic roles for the CPSR as well as factors that must be controlled during testing.

REMEMBERED INTENSITIES OF TASTE AND ORAL BURN CORRELATE WITH PROP BITTERNESS

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The bitterness of PROP (6-n-propylthiouracil) sorts subjects into non-tasters, medium tasters and supertasters. Subjects who had not previously tasted PROP (N=245) rated remembered sensations using the general LMS, the Labeled Magnitude Scale with the label "strongest imaginable sensation of any kind" at the top (Green et al, 1993; Bartoshuk et al, 2001). The sensations rated included brightest light seen, loudest sound heard, strongest saltiness, sweetness, sourness and bitterness tasted and strongest oral burn (eg, capsaicin) and oral pain (eg, toothache, canker sore) felt. PROP bitterness was rated on the same scale at the end of the experiment. All four strongest tastes correlated with PROP bitterness. Bitterness produced the highest correlation as it does when stimuli are actually tasted. Oral burn (most intense for supertasters) minus oral pain correlated with PROP bitterness. This explains why PROP effects are truncated using the original LMS; the "strongest oral sensation" is not equal to all. The brightest light did not correlate with PROP bitterness but the loudest sound did (albeit only slightly). This suggests that using sound as a control for PROP studies may cause the size of PROP effects to be slightly underestimated. We conclude that remembered sensations can reflect the markedly different orosensory worlds associated with PROP tasting. Supported by grant NIH DC00283.

DO INDIVIDUAL ODOR DETECTION THRESHOLDS PREDICT SUPRATHRESHOLD SCALING DATA?

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Individual variability in odor detection threshold is well-described and tends to follow a log-normal distribution. Variability in the rating of suprathreshold stimuli has been less studied, particularly in relationship to individual odor detection thresholds. We hypothesized that individuals who have greater odor detection acuity will also exhibit a steeper psychophysical function when rating suprathreshold stimuli. To test this hypothesis, 16 volunteers (evenly divided by gender; mean age 36 years) were tested on two occasions, at least a half-hour apart. The first task consisted of an odor detection threshold for n-propanol, using an ascending series, forced-choice, method of limits. The second task consisted of suprathreshold scaling of n-propanol using the method of magnitude estimation (four trials in varying order). The slope of the psychophysical function was defined as the difference in an individual's rating between the reference stimulus (fixed modulus = "10") and the stimulus two concentration steps stronger, divided by two (i.e., rating units per dilution step). A scattergram was then constructed comparing individual detection thresholds and psychophysical slopes. Finally, a regression line was drawn through these points. Although the slope of this line was in the hypothesized direction (0.52 rating units per dilution step^2), the slope was not significantly different from zero ($p=0.29$). Within the limits of our sample size, these data do not support the original hypothesis linking individual psychophysical function slopes with individual odor detection acuity.

GENERALIZATION OF CONDITIONED TASTE AVERSION BETWEEN SACCHARIN AND MONOSODIUM GLUTAMATE IN RATS

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Monosodium glutamate (MSG) has a unique taste that the Japanese call umami. Previous studies (Stapleton et al., Chem. Senses 24: 449-457, 1999; Yamamoto et al., Physiol. Behav. 49: 919-925, 1991) have found that in rats a conditioned taste aversion (CTA) to the taste of MSG mixed with amiloride generalized to the taste of sucrose. Amiloride reduces the taste of the Na+ component of MSG. CTA was used to determine whether the taste of saccharin would generalize to MSG. CTA procedures were conducted in a computer controlled Dialog Lickometer. Half of the rats were conditioned with LiCl while the rest received NaCl injections (IP) immediately after ingesting 1.25 mM saccharin. On test day, the rats were presented with saccharin (0.625 and 1.25 mM), MSG (10, 25, and 100 mM), KCl (25 mM), sucrose (100 mM), and water. All solutions contained 30 ìM amiloride. The rats showed a generalization of CTA to the taste of 100 mM sucrose and 100 mM MSG. The findings further support the hypothesis that MSG in the presence of amiloride has a sweet component similar to that of saccharin that is detectable by rats. Supported by NSF Grant IBN-9982913 awarded to ERD.

EFFECTS OF LABELED MAGNITUDE SCALE INSTRUCTIONS ON TASTE PERCEPTION OF NA-SACCHARIN, ACESULFAME-K AND 6N-PROPYL 2-THIOURACIL

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Specific instructions given to subjects regarding the upper endpoint of labeled magnitude scales (LMS) may affect ratings. Support for this observation comes from the evidence of contrast effects in many different sensory modalities (see Green et al. 1996, Chem. Senses 21, 323). The present experiments tested the supposition that a change in instructions regarding the upper endpoint of the LMS has an effect on the ratings given to two high intensity sweeteners by people of different 6n-propyl 2-thiouracil (PROP) taster status. A total of 61 subjects participated in one of two experiments. In the first experiment, the upper endpoint of the LMS was defined as "strongest imaginable oral sensation," while in the second experiment, it was defined as "strongest imaginable sensation of any kind." Subjects rated Na-saccharin, Acesulfame-K and PROP for sweetness, saltiness, sourness and bitterness sensations. The magnitudes of ratings and interactions between PROP taster groups and bitterness ratings were similar in both experiments. Significant positive correlations were found between bitterness ratings of the two PROP stimuli and between bitterness ratings of the higher levels of the two sweeteners. Principal components analysis applied to both data sets separately revealed factors accounting for the variances associated with general intensity, PROP bitterness and bitterness of the high-intensity sweetener stimuli. The similarities between these two studies suggest that variations in instructions did not affect the use of labeled magnitude scales.

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DISCRIMINATION BETWEEN THE TASTES OF N-METHYL-D-ASPARTATE AND MONOSODIUM GLUTAMATE IN RATS

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Monosodium glutamate (MSG) is a naturally occurring amino acid found in protein-rich foods such as meat, vegetables, and dairy products, and elicits a taste called *umami.*It has been hypothesized that MSG taste is transduced by N-methyl-D-aspartate (NMDA)-like receptors. However, molecular biological work and conditioned taste aversion (CTA) experiments by Chaudhari *et al*(J. Neurosci., 1996) indicated that MSG is transduced instead by taste-mGluR4 receptors. Indeed, a CTA to MSG does not generalize to NMDA, nor does an aversion for NMDA transfer to MSG in the presence of amiloride (Stapleton *et al,*Chem. Senses, 1999). To explore this question further, a shock-avoidance/water-reinforcement discrimination procedure was used to test if rats could discriminate between NMDA and MSG. If rats can discriminate between the two tastes, then this implies that MSG taste is not transduced solely by NMDA receptors. The first experiment showed that the threshold for NMDA was between 2.5-5 mM. Stapleton *et al*(AChemS Abs., 2000) found that the threshold for MSG is 1-3 mM. In the second experiment, rats were required to discriminate between MSG and NMDA across a range of concentrations (5-100 mM) in the presence of 30μM amiloride. Rats reliably distinguished between NMDA and MSG at 10 mM and higher. These results suggest that MSG transduction involves more than just NMDA receptors. Supported in part by NIH/NIDCD DC03013 (SDR).

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ASSESSMENT OF THREE METHODS FOR PROP TASTER STATUS CLASSIFICATION

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With respect to sensitivity to the perceived bitterness of 6-n-Propyl-2-thioUracyl (PROP), individuals have been commonly classified as Non Tasters (NT), Tasters (T), and Super Tasters (ST). This classification is an important segmentation tools for studies of food preferences and dietary choices, with yet mixed and contradictory results that could come from the lack of reliability of the classification methods themselves. The present work compares three methods and discusses their value in term of reliability and ease of use. In the first study, subjects (n=194) rated the perceived intensity of three different solutions of PROP and three of sodium chloride (NaCl). In the second study a subset of the same subjects (n=121) rated one solution of PROP and one of NaCl. Both studies were conducted in two replicates and ratings were made using the Labeled Magnitude Scale (LMS). Based on the results within each method, subjects were classified into group of NT, T and ST. The internal reliability is 72% and 80% respectively for the first and the second method. The reliability between the methods is 37%. However, it reaches 79% when classifying the subjects into only two groups: NT and other (T or ST). In the third study we investigated a method in which subjects indicated a difference in perceived intensity of 1 concentration of PROP from a reference stimulus (NACL). Difference scores were calculated for each subject and were used to classify subjects into PROP taster group. The reliability of this method and comparison with the classifications obtained with the other two methods will be presented and discussed.

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SELF- AND CROSS-ADAPTATION AMONG FOUR ODORANTS

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We are investigating the effects of cross-adaptation on the sensory properties of four odorants: linalyl acetate (LA), 2-undecanone (UN), 2,2,5-trimethyl-5-pentyl cyclopentanone (aka, veloutone, or VE), and a mixture of linalyl acetate and undecanone (LA-UN). In Study 1, 15 subjects rated the perceived intensity of three concentrations of VE after adaptation to LA, UN, VE, and a blank (BL). Relative to the blank, LA, UN, and VE caused statistically significant reductions in perceived intensity (ranking of average ratings: BL>LA>UN=VE). In Study 2, 15 subjects rated the perceived intensity of three concentrations of LA-UN after adaptation to LA, UN, LA-UN, and a blank. Relative to the blank, LA, UN, and LA-UN caused statistically significant reductions in perceived intensity (ranking of average ratings: BL>LA=UN>LA-UN). In Study 3, 15 subjects rated the perceived intensities of 1) three concentrations of LA, and 2) three concentrations of UN after adaptation to LA, UN, and a blank. Relative to the blank, LA and UN reduced the perceived intensities of LA and UN. In both cases, self-adaptation caused a greater reduction than cross-adaptation. The magnitude of cross-adaptation was approximately symmetric. The pattern of results suggests that both stereochemical and perceptual similarity play roles in determination of the functional similarity revealed in cross-adaptation.

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TASTE PROPERTIES OF CALCIUM SALTS AND MIXTURES

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Calcium fortification of processed foods is an increasingly common practice to enhance nutritional value. However, little has been published concerning the taste properties of divalent salts, including calcium. A series of psychophysical studies investigated the taste properties of calcium chloride, calcium lactate, magnesium sulfate and magnesium chloride. Divalent salts were characterized primarily by bitter taste (predominant above .032M), with additional sensations described as salty, metallic, astringent, sour and sweet, in decreasing order of intensity. Mixtures with sucrose, citric acid or sodium chloride with calcium chloride but suppressed bitter and metallic tastes. Calcium lactate, calcium gluconate and calcium glycerophosphate had lower salty and bitter responses than equimolar concentrations of calcium chloride, an effect suggesting anionic inhibition.

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THE TRANSCELLULAR TRANSDUCTION PATHWAY IS SUFFICIENT FOR NA+ RECOGNITION FOLLOWING ACUTE NA+ DEPLETION

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Large anions limit Na+ transduction through the paracellular transduction pathway without blocking the transcellular pathway. Previously, we showed the detectability function for Na+ salts to be independent of anion size, suggesting that the transcellular transduction pathway is sufficient for Na+ detectability in the rat. Although the paracellular transduction pathway is less sensitive to low Na+ concentrations than the transcellular pathway, its function in Na+ recognition has not been assessed. Recognition requires an accurate perception of taste quality in addition to detection of the stimulus, making it possible that the paracellular pathway is necessary for Na+ recognition. We tested whether inhibiting the paracellular Na+ transduction pathway with large anion Na+ salts compromised the rat's ability to recognize these salts as containing Na+ when in a Na+ deficient state. Sodium-deficient rats increased licking to all Na+ solutions in a brief access test, but not to nonsodium chloride salts. Thus, the appetite was specific to Na+ salts and not affected by the anion. When the transcellular pathway blocker amiloride was added to the salt stimuli, Na+-deficient rats increased licking of all salts regardless of cation or anion. These results support previous findings that the transcellular pathway is necessary for sodium recognition in the rat, and moreover, suggest that this pathway is also sufficient to perform this sensory function.

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EFFECTS OF CHLORHEXIDINE ON THE TASTE OF A SERIES OF SALTS

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Chlorhexidine, a bis-cationic biguanide antiseptic, causes temporary reductions in the perceived taste intensities of NaCl and quinine HCl. To address the specificity of these taste modifications, 12 subjects rated the intensity and identified the quality of 14 salts: 0.1M NaCl, 0.11M NaBr, 0.1M Na_2SO_4, 0.32M Na_3Citrate, 0.32M $NaNO_3$, 0.1M $NaHCO_3$, 0.032M NH_4Cl, 0.1M KCl, 0.1M LiCl, 0.1mM quinine HCl, 0.05mM quinine$_2SO_4$, 0.3M $MgCl_2$, 0.3M $MgSO_4$, and 0.032M $CaCl_2$. A sip and spit procedure was used and duplicate ratings were made before and after rinsing for two minutes with Peridex,® a mouthrinse containing 1.34mM chlorhexidine digluconate. This treatment reduced perceived intensities of halide and sulfate salts with monovalent cations ($p < 0.01$), but not those with divalent cations. Intensities of sodium nitrate, bicarbonate, or citrate were not affected. Salty identifications decreased ($p < 0.0001$) and sour and bitter identifications increased ($p < 0.0001$) for stimuli identified as "salty" pre-treatment. The quinine salts, reduced in intensity post-treatment, were primarily bitter before and after treatment. The magnesium and calcium salts, also primarily bitter, were unchanged in either intensity or quality by treatment. We hypothesize that chlorhexidine, a large, cationic compound with a strong affinity for oral tissue, interferes with ion channels involved in salt stimulus reception. Supported by UCHC Research Advisory Committee and NIH grant T35 DE07136.

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

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NaCl detection thresholds were determined for 12.5 and 50 mm^2 lingual areas at 4 anterior tongue locations in 8 subjects using the Regional Automated Taste Testing System (RATTS). The locations were the right tongue tip, an area 1.7 cm posterior to the tongue tip, and regions 1.7 and 3.4 cm posterior to the tip along the tongue's lateral margin. Stimulus duration was 0.75 sec. Thresholds were established using a two-alternative forced-choice single staircase procedure, and the fungiform papillae at each stimulation site were counted with the aid of videomicroscopy. NaCl thresholds were lower for the 50 mm^2 than the 12.5 mm^2 stimulation area at all target sites, and were directly related to papillary number among and within the stimulated regions. For a given number of papillae, thresholds were lower within the 12.5 mm^2 than within the 50 mm^2 stimulation region, likely reflecting taste bud density and activation of common afferent pathways. The tongue tip was more sensitive than any other tongue region, and the lateral margins were seemingly more sensitive than the lingual centrum. Large individual differences in taste sensitivity and tongue papilla numbers were noted, and some subjects were insensitive to the highest tastant concentrations at the non-tip loci. This study demonstrates that NaCl detection sensitivity varies across discrete regions of the anterior tongue and is related to the relative number and density of fungiform papillae. Supported by PO1 DC 00161 from the NIDCD, Bethesda, MD USA.

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CETYLPYRIDINIUM CHLORIDE IS BOTH AGONIST AND ANTAGONIST OF THE RAT AMILORIDE-INSENSITIVE CHORDA TYMPANI RESPONSE TO NACL, KCL, AND NH_4CL

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Rat chorda tympani (CT) responses to NaCl can be dissected into amiloride-sensitive (AS) and amiloride-insensitive (AI) components. An AS component is evidence for ENaC as a possible Na^+ taste receptor, a conclusion also confirmed by other approaches. Less is known about the AI component in part due to its unknown pharmacology. We now show that cetylpyridinium chloride (cpc) is both agonist and antagonist of the AI. CT responses to 0.1 M NaCl were enhanced by cpc from 50 μM to 250 μM cpc. Between 250 μM and 700 μM responses declined again, and at 1 and 2 mM cpc NaCl responses were inhibited. Pretreatment of the tongue with 250 μM cpc increased the response to 0.1 M NaCl by 80%. The half-time for return to control was 5 min. Pretreatment with 2 mM cpc inhibited the response by 40% with similar recovery kinetics. A mixture of 2 mM cpc + 100 μM amiloride, and NaCl from 50 - 500 mM eliminated the AI completely. Responses to 0.1 M KCl and 0.1 M NH_4Cl were also enhanced by 250 μM cpc and suppressed by 2 mM cpc. The influx of NH_4^+ across the apical membranes of polarized taste receptor cells (TRCs), measured as the rate of decrease in intracellular pH, was inhibited by cpc. The results suggest that a non-specific cation channel is present in the apical membrane of TRCs that mediates the AI of the NaCl response. The AI is also involved in responses to KCl and NH_4Cl. Supported by NIH grants DC00122, DC02422, Dept. of Veterans Affairs, and GlaxoSmithKline.

SALT INTENSITY AND BEHAVIORS: ASSOCIATIONS WITH BITTERNESS OF 6-N-PROPYLTHIOURACIL (PROP)

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We tested the relationship between PROP bitterness and salt intensity and preference in 38 females and 39 males who reported low cognitive restraint over eating. Subjects used the general labeled magnitude scale (Green et al, 1993; Bartoshuk et al, in press) to rate: the saltiness of NaCl in deionized water and chicken broth; the degree of liking/disliking for chicken broth with added salt; and the bitterness of 3.2 mM PROP. Subjects also reported how often they added salt to foods (usually, sometimes, hardly ever). Through Pearson correlation analyses, those who rated PROP as more bitter also rated the intensity of 1 M salt in solution and 1.12 M salt in chicken broth as more intense. Those who tasted PROP as more bitter also rated less liking for the 1.12 M NaCl in chicken broth. In chi square analyses, reported addition of salt to food differed between nontasters (PROP as less than "moderate"), medium (PROP between "moderate" and "very strong"), and supertasters (PROP as greater than "very strong"). Nontasters were significantly more likely to report "usually" adding salt to food than were supertasters. The sensory results extend previous findings (Bartoshuk et al, 1998) to PROP effects on salt tasting in a soup. The intake data suggest that this sensory difference could influence salt behaviors. (NRICGP/USDA 9603745 and NIH DC00283 supported).

AMILORIDE-SENSITIVE COMPONENT OF THE CHORDA TYMPANI NERVE RESPONSE REDUCED BY LOW NACL DIET IN ADULT RATS

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Previously, we have reported that a low perinatal NaCl diet maintained through day 30 produced no long-term effects on the responsiveness of the chorda tympani (CT) nerve to salts. Here we examine the influence of maintained low or high perinatal NaCl diets on CT response to salts. Different groups of rats were raised on either Basal (0.1%), Intermediate (1%) or High (3%) NaCl diets from conception until testing in adulthood (>90 days). Whole nerve electrophysiological recordings were obtained from the CT of the adult rats during stimulation of the anterior tongue with increasing concentration series of NaCl, KCl, and Q-HCl with and without 100 μM amiloride in the solution. While the relative response magnitudes of Q-HCl did not differ between groups, the relative response for NaCl and KCl was smaller for the Basal group than the Intermediate and High groups, which were similar. Amiloride suppressed the NaCl response more effectively for the Intermediate (28%) versus the High group (18%). There was no effect of amiloride on the NaCl response for the Basal group. There was a smaller but similar amiloride suppression of the KCl response for the Intermediate and High groups (10% inhibition). There was no amiloride inhibition of Q-HCl in any of dietary groups. There was no observable amiloride-sensitive component to the NaCl response in rats maintained on the Basal NaCl diet from conception to adulthood. This research supported by NIH Grant DC 02641.

OMP IS A BETA-CLAM

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OMP is an abundant, cytoplasmic protein present in mature olfactory sensory neurons of vertebrates from fish to humans. Its primary sequence is phylogenetically conserved and is 55% identical from Xenopus to humans. OMP has no homologs in any database, nor have any functional domains been identified in its primary sequence. Evidence for OMP's functional role in sensory transduction derives from both electrophysiological (Buiakova et al.'96, Ivic et al. '00) and behavioral deficits (Youngentob et al.'99) seen in OMP-KO mice. We hypothesize that the 3D structure of OMP conveys information not obvious in the amino acid sequence. We have determined the 3D solution structure of OMP (Baldisseri et al. '00) by heteronuclear, multi-dimensional NMR spectroscopy of singly, doubly and triply isotopically labeled OMP. Its structure consists of 8 beta-strands and 2 alpha-helices that form two nearly perpendicular beta-sheets and a hydrophobic core. This 3D structure most closely matches a beta-clam fold with a cleft/canyon separating the 2 beta-sheets providing a potential binding surface. Titrations with a peptide derived from a recently identified binding partner (Behrens et al. unpub.) indicate that the peptide binds in the cleft/canyon, confirming OMP's involvement in protein/protein interaction. This report comprises the first NMR structural analysis for any protein involved in chemosensory transduction and provides insight into OMP function. Supported by DC03112, DC00347 (FLM) and DC04752 (DMB).

WHY DO ODORANT BINDING PROTEINS BIND ODORANTS?

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We have investigated the properties of soluble proteins that interact with Z7-dodecen-1-yl acetate (Z7-12:Ac), the sex pheromone of the Asian elephant. Our previous experiments showed that the pheromone is excreted in the female urine bound to albumin, and released from the albumin upon pH change caused by contact with trunk mucus. We have found an abundant 20 kDa protein in the mucus of the trunk, homologous to known odorant binding proteins (OBPs), that binds the pheromone. A series of experiments has been carried out to ascertain the roles of the albumin and the OBP in transport and delivery of the pheromone to the receptor cell. Using the volatile odorant binding assay we have determined the dissociation constant of the OBP/pheromone complex. Using several methods we have been able to estimate the rates of association and dissociation of the OBP and the pheromone. Our results indicate that the binding of the pheromone, as well as of other odorants, by the OBP is too slow to play a significant role in the delivery of the ligand to the receptor. We also present evidence showing that even lipophilic odorants may travel across the mucous layer to the receptor cell without an assistance from a binding protein. Our results suggest that the main function of mammalian OBPs is to sequester excess ligands, rather than to deliver ligands to the receptor. Since Z7-12:Ac is also a common insect pheromone, the relevance of our results to insect systems is discussed. Supported by NIH grant RO1-DC03320 (to L.E.R.).

161 Slide XX. Molecular Biology of Olfaction

ATP EVOKES CA^{2+} INCREASES AND INWARD CURRENTS IN MOUSE OLFACTORY RECEPTOR NEURONS

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Purinergic nucleotides are important neuromodulators of auditory and visual systems. Purines are potent chemical stimuli for olfactory receptor neurons (ORNs) in catfish and lobster, however, the purinergic pathways in the mammalian peripheral olfactory system have not been studied. Application of ATP (10μM) onto perforated patched mouse ORNs evoked inward curents with two distinct latent periods. Some cells exhibited a long latent period suggesting that G-protein coupled P2Y receptors are expressed in mouse ORNs. Rapid activation of inward current with little or no desensitization was also observed, suggesting involvement of a ligand-gated P2X receptor. Application of ATP (10μM) evoked a rapid transient increase in intracellular calcium ($[Ca^{2+}]_i$) (76/84 cells; mean maximum increase = 151 ± 12%). In the absence of external Ca^{2+}, the ATP-induced rise in $[Ca^{2+}]_i$ was 31 ± 11% larger than responses in the presence of Ca^{2+} suggesting that at least part of the signal results from release from intracellular Ca^{2+} stores, implicating P2Y receptor contribution to ATP-mediated Ca^{2+} transients. An olfactory epithelial (OE) slice preparation and confocal imaging was used to measure changes in fluorescence intensities in fluo-4 AM loaded OE slices in response to odor, ATP, or odor + ATP. In most cells, ATP reduced the summed odor-induced changes in Ca^{2+}, however, two cells exhibited an increase in evoked $[Ca^{2+}]_i$ increase, suggesting a synergistic effect. This indicates that ATP modulation of odor responses may be dependent on the subtype(s) of P2 receptors expressed. This research was supported by NIDCD DC02994.

162 Slide XX. Molecular Biology of Olfaction

THE CYCLIC NUCLEOTIDE-GATED CHANNEL SUBUNIT OCNC2 IS ESSENTIAL FOR OLFACTORY ADAPTATION

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Sensory adaptation is critical for retaining a high degree of sensitivity and for preventing the saturation of the transduction machinery under continuous or repetitive stimulation. Short-term olfactory adaptation is known to be Ca^{2+}-dependent and has been hypothesized to involve modulation of the cyclic nucleotide-gated channel (CNC). Here we report that the deletion of one of the olfactory CNC subunits, OCNC2, nearly abolishes odor-dependent adaptation. We created an OCNC2-null mouse through homologous recombination in embryonic stem cells. OCNC2 message is undetectable in olfactory receptor neurons (ORNs) of null mice; other transduction components appear to be expressed at normal levels. The mutant CNC exhibits a decreased sensitivity to cAMP. Most interestingly, ORNs in OCNC2-null mice exhibit both a significantly reduced adaptation onset rate and a significantly increased rate of recovery from adaptation. These profound effects may be due to a change in the kinetics of Ca^{2+}/calmodulin interaction with the CNC. These data demonstrate that OCNC2 plays an essential role in mediating the Ca^{2+}-dependent adaptation of ORNs, making it the first gene to be directly implicated in olfactory adaptation. Supported by HHMI (RRR), NIDCD (RRR) & NINDS (FZ).

163 Symposium XXI. Seeing is Believing: Imaging Cellular Function in the Chemical Senses

INTRODUCTION TO "SEEING IS BELIEVING: IMAGING CELLULAR FUNCTION IN THE CHEMICAL SENSES"

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New methodologies generate new ideas. The recent development of novel imaging techniques in the chemical senses has shed a bright light on existing questions and led to new understanding in gustation and olfaction. This symposium features 3 exciting approaches to studying taste and olfaction and introduces 3 enthusiastic young investigators who are beginning to have a major impact in the field. The talks will describe different imaging methodologies and provide us with some of the latest findings that stem from these approaches.

164 Symposium XXI. Seeing is Believing: Imaging Cellular Function in the Chemical Senses

VISUALIZING RESPONSES TO NEUROTRANSMITTERS AND TASTE STIMULI IN TASTE CELLS

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In the past, it has been very difficult to examine sensory and synaptic responses in taste cells in the intact tongue, mostly due to technical limitations. For instance, there is no ready access to taste cells within taste buds for electrophysiological recordings. We have developed a slice preparation of rat foliate papillae and a new Ca^{2+} microfluorometric technique to measure changes in intracellular $[Ca^{2+}]$ induced by stimulation of taste cells *in situ* . Changes in $[Ca^{2+}]$i can be detected over several hours in response to chemical stimulation, and tissue integrity and potential cell-cell synaptic connections in taste buds are preserved. We can measure $[Ca^{2+}]$i changes in several taste cells and several taste buds simultaneously in response to different stimuli. We initially used our new technique to study activation of neurotransmitter receptors in taste cells. Taste cells responde to glutamate in a concentration-dependent manner. Pharmacological characterization of the responses revealed that there are synaptic glutamate receptors of the non-NMDA in taste cells. In another study, we investigated how bitter taste stimuli are detected and signals encoded in taste buds. Two-thirds of the bitter-sensitive cells respond selectively to only one bitter compound of those tested. The remainder respond to 2 or more stimuli. These results suggest that taste cells can be somewhat selective in their sensitivity to taste stimuli. As shown by these results, our imaging approach provides a new tool to address many open issues in gustatory physiology.

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OPTICAL IMAGING OF INTRINSIC SIGNALS IN THE OLFACTORY BULB

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Optical imaging of intrinsic signals enables one to visualize stimulus evoked patterns of activity in many sensory cortices and has revealed previously unseen features. We have applied this technique to the rodent olfactory bulb to examine the patterns of activity evoked by olfactory stimuli. The high spatial resolution of the technique enables us to visualize individual and groups of activated glomeruli. Consistent with prior physiological and molecular biological investigations, different odorants activate distinct sets of glomeruli, and different glomeruli can be activated by distinct sets of odorants. Increasing odorant concentration increases the number of active glomeruli. The patterns of activated glomeruli are variable, but generally similar between animals and bilaterally symmetrical in the two bulbs of individual animals. Because intrinsic signal imaging is relatively noninvasive, imaging can be combined with anatomical, physiological, and behavioral experiments. To investigate the link between odor-induced activity in the olfactory bulb and odor perception, we have correlated the pattern of activated glomeruli seen by imaging with behavioral assessment of perception. We focused on the representation of enantiomers—pairs of mirror-symmetric, nonsuperimposable molecules that share nearly every chemical property. Some enantiomers, such as (+)- and (-)-carvone, are readily distinguishable while others, such as (+)- and (-)-2-butanol are rarely discriminated. In contrast to humans, rats behaviorally discriminate both types of enantiomer pairs. Imaging reveals glomeruli selective for each enantiomer pair member. These findings indicate that the pattern of activated glomeruli provides sufficient information to discriminate molecular shape.

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FUNCTIONAL IMAGING OF THE HUMAN BRAIN: WHAT HAS IT TAUGHT US AND WHAT CAN IT TEACH US ABOUT OLFACTION?

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One of the most exciting developments in neurobiology over the past decade has bee the advent of noninvasive methods for visualizing neural activity in humans in vivo. The most popular of these methods are positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Both of these methods have been applied to the study of human olfaction, but more often than not, have failed to enable measurement of odorant induced activity in primary olfactory cortex. We will review work from several laboratories, including ours, to provide a current picture of the methods, findings, and problems facing the field. We will show that odorant induced activity in primary olfactory cortex has often gone undetected with functional imaging because of the rapid time-course of habituation in primary olfactory cortex. We will present an experimental design, termed **event-related functional imaging,**that enables consistent measurements of odorant induced activation in human primary olfactory cortex despite this rapid habituation. Finally, we will describe potential future directions for functional imaging of human olfaction. We propose that functional imaging may merit a redefinition of human primary olfactory cortex, based on functional rather than structural criteria.

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PERIPHERAL GUSTATORY SYSTEM IN BDNF/NT-3 DOUBLE KNOCKOUT MICE

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We have previously shown that the neurotrophic proteins brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) are expressed in lingual papillae and play crucial roles for the lingual gustatory and somatosensory innervation, development of taste buds (downstream to gustatory innervation), and preserving gustatory papillae morphology. We have also shown that BDNF null-mutated mice exhibit gustatory deficits while lingual somatosensory innervation of the tongue is disturbed in NT-3 knockouts. Here, we have generated BDNF/NT-3 double knockout mice and have studied the tongue phenotype in these animals. BDNF/NT-3 mice were generated by crossing breeding pairs of BDNF and NT-3 heterozygous mice. BDNF/NT-3 KO mice die at birth and we therefore focused our present study on newborn animals. A midline vallate papilla and a few fungiform papillae were present in the double KO mice. Vallate papilla morphology was distorted. There were no intraepithelial nerve fibers in the vallate gustatory epithelium, and vallate subepithelial nerve plexus was reduced in size. The few remaining fungiform papillae were significantly reduced in size and had a scarce innervation, but nerve fibers were always present in these remaining papillae. Interestingly, the remaining ventral surface fungiform papillae were morphologically different from dorsal surface papillae. Our findings show that BDNF and NT-3 support distinct sensory modalities in the tongue.

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PREPARATION AND INITIAL CHARACTERIZATION OF MICE WITH TARGETED DELETION OF THE OLFACTORY MUCOSA-SPECIFIC *CYP2G1* GENE

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CYP2G1 is an abundant P450 enzyme expressed uniquely in the olfactory mucosa of mammalian animals. CYP2G1 has high metabolic activities toward sex steroid hormones, odorants, as well as olfactory toxicants. To evaluate the possible roles of CYP2G1 in cellular homeostasis, odorant clearance, and olfactory mucosa-specific toxicity of xenobiotic compounds, we have generated *Cyp2g1*-null mice by replacing exon 3 of mouse *Cyp2g1* gene with the *neo* gene. Homozygous *Cyp2g1*-null mice are viable and fertile. There is no evidence of embryonic lethality or developmental deficits. No morphological changes or histological abnormalities were detected in the olfactory mucosa, brain, heart, lung, kidney, liver, small intestine, and testis of the $Cyp2g1^{-/-}$ mice. Northern blot analysis confirmed that the levels of CYP2G1 transcripts in the olfactory mucosa are reduced in $Cyp2g1^{+/-}$ mice, and are not detectable in the $Cyp2g1^{-/-}$ mice. Compared with wild-type C57BL/6 or SvJ129 mice, $Cyp2g1^{-/-}$ mice exhibited about 75% lower activities toward testosterone in olfactory mucosal microsomes at a substrate concentration of 5 uM. This confirms that CYP2G1 plays a major role in testosterone metabolism in mouse olfactory microsomes. Further investigations are under way to determine the contributions of CYP2G1 to nasal microsomal metabolism of other steroid hormones, odorants, and nasal toxicants, and to examine its roles in xenobiotic toxicity in the olfactory mucosa. (Supported in part by NIH grant ES07462).

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WHAT MAKES A SUPERTASTER?

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Bitterness of PROP (6-n-propylthiouracil) varies genetically. To nontasters (two recessive alleles), PROP is virtually tasteless. Tasters can be divided into those who taste the most (supertasters) and least bitterness (medium tasters). On average, supertasters perceive the most intense tastes (bitter substances tend to show the greatest differences across the three groups), the most intense oral burn from irritants, the most intense touch sensations from substances that thicken foods (eg, fats); they also have the most fungiform papillae. This provides some insights into the genetics of supertasting. Nontasters have fewer fungiform papillae than tasters; this shows that genes for PROP bitterness and anatomy cannot be independent. Supertasters cannot simply be tasters who have a large number of fungiform papillae because the bitterness of PROP shows much greater variation across the three groups than does any other tastant. We suggest that supertasters are both homozygous for the dominant PROP gene and have a large number of fungiform papillae. The greater perceived intensities of many non-PROP oral stimuli may be produced by greater numbers of fungiform papillae. Factors that alter taste without altering number of fungiform papillae (eg, pathology, sex hormones) complicate establishing the relative contributions of genes mediating PROP tasting and fungiform papillae number. Genetic testing will clarify this. Funded by NIH DC00283, NRICGP/USDA 9603745.

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FUNGIFORM PAPILLAE AND CIRCUMVALLATE TASTE BUDS ARE LOST IN MICE LACKING THE NEUROTROPHIN RECEPTOR p75

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Gustatory neurons and taste buds depend on the neurotrophins, brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT4), for their survival and development. The tyrosine receptor kinase, TrkB, is an important receptor for these neurotrophins and it's absence severely impairs gustatory system development. However, the p75 receptor has also been implicated in regulation of the binding and transport of BDNF and NT4. To determine if the p75 receptor is important for normal gustatory development, the number of fungiform papillae and number of circumvallate taste buds were quantified in mice that lack p75 ($p75^{-/-}$). The tongues of $p75^{-/-}$ mice had 39% fewer fungiform papillae than littermate controls (n=3, $p<0.04$). The circumvallate papilla was smaller and contained 35% fewer taste buds in $p75^{-/-}$ mice compared to controls (n=3, $p<0.01$). Thus, the p75 receptor is required for the development of a full complement of fungiform and circumvallate taste buds. Whether the observed taste bud loss corresponds to a similar decrease in gustatory neurons in $p75^{-/-}$ mice is now being determined. This work is supported by F32 DC00370 (RFK) and NS 33730 (KMA).

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HIGH-RESOLUTION GENETIC MAPPING OF THE *SOA* LOCUS

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Sucrose octaacetate (SOA), an acetylated sugar, tastes bitter to humans and has an aversive taste to some other species. In mice, behavioral acceptance of SOA depends on allelic variation of a single locus, *Soa*. Three Soa alleles determine¬®taster¬-(*Soa*^{a}),¬®nontaster¬- (*Soa*^{b}) and ¬®demi-taster¬- (*Soa*^{c}) phenotypes of taste sensitivity to SOA. Although *Soa* has been mapped to distal chromosome six, the limits of the *Soa* region have not been defined with high resolution. In this study, mice from congenic strains SW.B6-*Soa*^{b}, B6.SW-*Soa*^{a} and C3.SW-*Soa*$^{a/c}$, and from outbred CFW strain were genotyped with polymorphic markers on chromosome 6. In the congenic strains, limits of donor fragments were determined. In outbred mice, linkage disequilibrium and haplotype analyses were conducted. Positions of markers were further resolved using radiation hybrid mapping. The results show that the *Soa* locus is contained within a ~1-cM(3.3-4.9Mb) region including the *Prp* locus. Key words: taste, bitter, mice, genetics.

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C57BL/6J AND C57L/J MICE DIFFER IN THEIR ACID TASTE SENSITIVITY: EVIDENCE FROM BEHAVIORAL AND PHYSIOLOGICAL STUDIES

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In two-bottle tests, C57L/J (L) mice avoid citric acid or HCl at higher concentrations than C5LBL/6J (B6) mice do. We sought to characterize this difference by means of a short-term behavior assay designed to minimize post-ingestive cues, as well as whole-nerve chorda tympani (CT) recordings. Following 24-h water deprivation, licking behavior (5 s trials) in L and B6 mice to concentrations of HCl and citric acid (1-100 mM) was compared to water lick rates. Both strains decreased licking with increasing acid concentration. However, L mice licked both acids at a higher rate than B6 mice, indicating that they may be less sensitive to the aversive taste of the acids. We next measured the whole-nerve CT response to both HCl (0.01 - 30 mM) and citric acid (0.01 - 100 mM) in these mice. Surprisingly, although the response thresholds did not notably differ between strains, there was a marked increase in relative response for L mice at concentrations > 10 mM. Application of amiloride or the Cl channel blocker NPPB did not affect responses to acids in either strain. These studies demonstrate a peripheral basis for strain differences in acid taste, but the exact nature of these mechanisms needs further studies. Support: NIH Grant DC03854 Grant to AB, DC00882 to GB, UMB Grant 01063 to JB.

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PTC TASTERS OUTPERFORM PTC NON-TASTERS ON A TEST OF REGIONAL (CN VII AND CN IX) TASTE IDENTIFICATION

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It has been suggested that the ability to taste phenylthiocarbamide (PTC), 6-n-propylthiouracil (PROP), and similar substances is largely an inherited trait. Recent studies suggest that such ability may not reflect a simple dichotomy, however, and is correlated with the number of lingual fungiform papillae. In this study, we determined whether traditionally defined "PTC tasters" differ from "PTC non-tasters" in general ability to identify suprathreshold concentrations of sucrose, citric acid, caffeine and NaCl presented to anterior (CN VII) and posterior (CN IX) tongue regions. These stimuli were equated for perceived viscosity and intensity. PTC tasters significantly outperformed non-tasters in identifying stimulants in all regions tested. The only stimulus for which PTC tasters did not perform significantly better than non-tasters was sucrose. Since most subjects identified the sucrose concentration employed in this study at nearly 100%, it is not clear whether this phenomenon is due to a ceiling effect or other factors. That being said, however, the data suggest that the ability to discern the concentration of PTC used in this study may be predictive of overall general taste function. Supported, in part, by Grants PO1 DC 00161, RO1 DC 04278, RO1 DC 02974, and RO1 AG 27496 from the National Institutes of Health, Bethesda, MD USA.

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NOTCH SIGNALING GENES EXPRESS IN DEVELOPING TASTE PAPILLA

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The Notch pathway is an evolutionary conserved cell-cell signaling mechanism involved in cell fate decisions during different cellular and developmental processes in invertebrates and vertebrates. Recent studies have disclosed that the Notch pathway is involved in determining cell fate in various sensory organs. However, little is known about the developmental mechanisms that regulate taste bud cell differentiation in lingual epithelia. To clarify the mechanisms that regulate taste bud cell differentiation in fetal lingual epithelia, we have investigated the expression patterns of Notch and its ligand Jaggeds, hairy/enhancer of split (Hes1), and a mammalian homolog of the achaete-scute complex (Mash1) in fetal and adult mouse tongues using in situ hybridization. Notch, Notch ligands and Hes1 are expressed in developing taste papillae. The expression of Notch1 resolves to perigemmal cells and basal cells of lingual epithelia. On the other hand, expression of Notch ligands and Hes1 resolves to subsets of taste cells within buds, and to basal cells of the lingual epithelia in adults. Mash1 is expressed broadly in the epithelium of developing papillae, but gradually resolves to a small subset of taste cells within adult taste buds. Thus, the Notch pathway is likely involved in determining cell fate in fetal tongue epithelium. Supported by NIDCD (DC03947) to LAB.

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SURVIVAL AND NEUROPHYSIOLOGY OF EMBRYONIC RAT GENICULATE GANGLION NEURONS ARE ALTERED BY NEUROTROPHINS

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Neurotrophins encountered by geniculate ganglion neurons, in and on their way to their anterior tongue taste papilla targets, have been proposed as molecular regulators of ganglion cell survival and differentiation, and neurite guidance. We hypothesized that different neurotrophins selectively alter survival and function of geniculate cells during target innervation. Embryos were removed from anesthetized pregnant rats at gestation day 16, a stage of dense innervation of the fungiform papillae. Ganglia were explanted onto matrix-coated coverslips and cultured in medium supplemented with 10 ng/ml BDNF, NT4, NGF or NT3. Cell survival was evaluated by counting neurons from 4-7 ganglia in each neurotrophin group after 5- 6 days in culture. Survival was best in cultures with BDNF or NT4, with over a thousand cells maintained. Survival was decreased by about 85% when cells were maintained with NGF or NT3. To study neurotrophin effects on cell function, whole cell recordings were made from ganglion neurons after 4-8 days in culture with BDNF (67 cells), NT4 (51), NGF (55) or NT3 (52). Ganglion cells cultured with BDNF or NT4 had similar passive membrane and action potential properties. Cells maintained with NGF or NT3 had increased excitability, and with NT3 had larger and sharper action potentials. Thus, both survival and electrophysiological properties of developing geniculate ganglion neurons are substantially and selectively modified by exposure to different neurotrophins. Supported by NIDCD, NIH Grant DC00456.

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ALPHA-GUSTDUCIN IMMUNOREACTIVITY IN DEVELOPING HAMSTER TASTE BUDS

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During postnatal development, hamster chorda tympani sensitivity to saccharides increases from low levels around 15 days of age to mature levels by about 120 days of age. Interestingly, mice that lack the gene for the receptor-linked GTP-binding protein subunit, alpha-gustducin, show abnormally low behavioral and electrophysiological sensitivity to 'sweet' stimuli. In the present study, we used immunohistochemistry to test the hypothesis that delayed postnatal expression of alpha-gustducin in suckling hamster fungiform papilla taste receptor cells might contribute to reduced neonatal sensitivity to sweet stimuli. Frozen sections of paraformaldehyde-fixed tongue were obtained from 5- and 9-day-old and adult hamsters, and incubated with antisera to rat alpha-gustducin. Bound primary antibodies were visualized by indirect immunofluorescence. Similar patterns of alpha-gustducin immunoreactivity were observed in adult and 9-day-old hamster fungiform papilla taste buds. In contrast, alpha-gustducin immunoreactivity was scant or absent in fungiform papilla taste buds of most 5-day-olds (80%, N=5). Thus, while taste cell alpha-gustducin expression appears to commence during the postnatal period, its timing indicates that alpha-gustducin expression alone cannot account for the later physiological appearance of chorda tympani sensitivity to 'sweet' stimuli in hamster. Supported by NIH Grant DC04271 and W&L R.E. Lee and Glenn Research Endowments.

A POSSIBLE LIGAND-BASED ROLE FOR NEUROPILIN-2 IN CRANIAL SENSORY NERVE PATHFINDING

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Neuropilins have dual roles: they are part of the receptor complex for diffusible axon guidance molecules (class 3 semaphorins, Sema3's), and they can mediate intercellular adhesion by binding to neuropilins in the membranes of adjacent cells. Neuropilin-1 and -2 are both expressed by rat geniculate and trigeminal axons during early pathfinding stages (E12-13), and Sema3A and Sema3F, which require neuropilin-1 and -2, respectively, repel axon outgrowth from these ganglia in vitro. Pathway explants derived from the hyoid and mandibular arches also repel this outgrowth, however this repulsion is blocked by anti-neuropilin-1, and not affected by anti-neuropilin-2 (Rochlin et al., J. Comp. Neurol. 422:579-593). This argues against a role for diffusible ligands of neuropilin-2 in guiding peripheral nerves at these stages. To determine if the distribution of neuropilins was compatible with a haptotactic role for neuropilin-2, wholemount immunolocalization of neuropilin-1 and -2 was undertaken. Anti-neuropilin-1 stained a meshwork of blood vessels, whereas neuropilin-2 labeling was concentrated in branchial arch arteries. Trigeminal and at least a subset of geniculate peripheral axons that entered the mandibular and hyoid arches, respectively, appeared to grow along these arteries. These data are consistent with a role for neuropilin-2 in haptotactic guidance of cranial sensory axons.

NEURON-TARGET MATCHING IN DEVELOPING TASTE BUDS IS ACCOMPLISHED BY INCREASES IN TASTE CELL NUMBER

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The chorda tympani nerve, whose cell bodies are contained within the geniculate ganglion, innervates taste buds in the anterior tongue. There is a significant positive correlation match between the size of individual taste buds and the number of neurons that innervate them in normal adult rats. Simply stated, in adults large taste buds receive innervation from many neurons, and few neurons innervate small taste buds. While the heterogeneous, mature pattern of innervation is established by postnatal day 10 (P10), taste buds are uniformly small. Therefore, the match between numbers of innervating ganglion neurons and taste bud size does not fully develop until P40, when taste buds reach their mature size (Krimm and Hill, 1998). To assess the developmental process further, stereological reconstructions of taste buds from rats aged ten and forty days were undertaken to assess the manner by which taste bud volume increase occur. Cytokeratin-19 positive taste receptor cells were counted from serial 1 μm optical sections. Taste buds from animals aged ten days contain an average of 15 cells, while adult taste buds contain an average of 50 cells. Thus, the developmental increase in taste bud volume is largely due to addition of taste receptor cells. It is possible that the maximum number of taste cells supported in each taste bud is dependent on the number of innervating neurons. Suppported by NIH grants DC00407 to D.L.H. and HD07232 to S.J.H.

PLACODALLY DERIVED SENSORY FIBERS GROW PREFERENTIALLY, BUT NOT EXCLUSIVELY, TOWARD OROPHARYNGEAL ENDODERM

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Axon pathfinding relies on the collective influence of attractive and repulsive cues. Given that taste buds in salamanders arise autonomously from the oropharyngeal endoderm, we aimed to test how gustatory axons pathfind to taste buds. We developed a method to grow sensory neurons in vitro from explanted epibranchial placodal tissue. These placodes produce sensory neurons of the VII, IX and X cranial ganglia, which are believed to innervate taste buds. First, we assessed axon growth patterns when cultured with oropharyngeal endoderm (OPE) which gives rise to taste buds; and flank ectoderm (FE), which these neurons do not encounter in vivo. Interestingly, using circular distribution statistics to analyze fiber growth, we found OPE and FE to be equally attractive. This might suggest that embryonic tissues produce a generic attractive cue. To test this hypothesis, we cultured neurons with two other targets: gut endoderm (not encountered in vivo) and notochord (not innervated). We found that fiber growth is random with respect to these tissues, implying a conserved mechanism may account for the attraction of placodal fibers to FE and OPE. Alternatively, the placode may give rise to a heterogeneous collection of neurons that respond to different cues from FE and OPE. We aim to assess this possible heterogeneity using a peptide marker to identify intragemmal fibers that would be expected to grow preferentially towards OPE. Supported by NIDCD (DC03947 & DC03128 to LAB).

CHARACTERIZATION OF WHOLE CHORDA TYMPANI NERVE TASTE RESPONSES DURING DEVELOPMENT IN THE C57BL/6J MOUSE

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Progress in research of the gustatory system is expediently becoming dependent on work involving genetically controlled models. Many unanswered questions about the mechanisms involved in taste development can be elucidated through molecular biological manipulations of well-characterized mouse strains. For example, the molecular mechanisms involved in the regulation and expression of salt taste development will likely be revealed by controlled manipulations of the underlying transduction pathways. Unfortunately, the best characterized model for taste transduction, the Sprague Dawely rat, is not easily genetically altered. It has become critical to identify a generally amenable strain of mouse whose gustatory response development most resembles those of the Sprague Dawely rat. As an initial step, we recorded chorda tympani responses to 50-500 mM NaCl, NaAc, KCl, and NH_4Cl in C57BL/6J adult and weanling mice. Preliminary results indicate that this mouse strain exhibits similar responses to the Sprague Dawley rat, whose taste responses have been well characterized as showing a developmental increase in sodium response. The C57BL/6J model is capable of serving as a background/wild type for future genetic manipulations, while potentially generalizing from previous studies using the Sprague Dawely rat. Supported by NIH grant DC00407.

WITHDRAWN

DISTRIBUTION OF CCK, CGRP, NPY, AND GALANIN IN THE PRIMARY GUSTATORY NUCLEUS OF THE GOLDFISH

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The primary gustatory nucleus of the goldfish, the vagal lobe, is a large laminated structure composed of discrete sensory, fiber, and motor layers. The vagal lobes receive primary afferent input from the gustatory portion of the vagus nerve and contain reflex circuitry involved in the ingestion or rejection of potential food items. In the present study we examined the distribution within the vagal lobe of four different neuropeptides implicated in the regulation of ingestion. Immunohistochemistry indicates a heavy concentration of CCK, CGRP, NPY, and galanin immunoreactive fibers in the capsular fiber layer as well as fibers running both parallel and perpendicular to deeper sensory layers. Immunoreactivity for all four peptides also can be detected in fibers at the periphery of the motor and deep fiber layers and within the soma of many motor neurons of the dorsal motor nucleus of the vagus. Capsular immunoreactivity for CCK and CGRP is greatly reduced one to two weeks following vagus nerve transection indicating that the majority of these fibers are from primary sensory afferents. In contrast, NPY and galanin immunoreactivity in the capsular fiber layer and reactivity for all four peptides in the deeper sensory and fiber layers is only modestly affected by vagal transection, indicating that these fibers are intrinsic to the central nervous system.

SEROTONERGIC MODULATION OF PUTATIVE IONOTROPIC GABA RECEPTORS IN CRAYFISH OLFACTORY PROJECTION NEURONS

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In the spiny lobster, olfactory projection neurons (OPNs) are known to possess ionotropic receptors for the inhibitory transmitter GABA (Zhainazarov et al., J Neurophysiol 76:2235 (1996). Here we report modulation by serotonin (5-HT) of GABA-activated current (I) in freshly isolated OPN somata from the freshwater crayfish . Using whole-cell perforated patch clamp recording techniques we found that 5-HT (1-1000 M) suppressed I in a majority of the cells examined (37/43;86%), while in a much smaller proportion of the OPNs (4/43;9.3%)it had no effect. In two cells 5-HT slightly potentiated I. 5-HT inhibited 1000 M GABA-activated currents in a dose-dependent manner. Our preliminary data suggest that this modulatory effect is non-competitive and non-voltage dependent. The variability in OPN responsiveness to 5-HT may reside in the diversity of functional types that constitute the cluster of OPN somata, which nonetheless are uniform in shape and cellular diameter. Our data suggest that 5-HT can increase the excitability in a majority of OPNs by suppression of GABA-induced currents, thereby regulating the efficacy of inhibitory synaptic connections. The underlying mechanism of serotonergic modulation of I may be caused by the phosphorylation of the GABA -receptor complex through activation of different serotonin receptor subtypes with individually diverse signal transduction pathways. Supported by NSF grant IBN-9727753. $\mu\mu$.

SUBSTANCE P AND GNRH COLOCALIZE IN THE NERVUS TERMINALIS OF SALAMANDER

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Previous studies have reported substance P-immunoreactivity (SP-ir) in nervus terminalis (NT) of several fish species. However, we have not observed SP-ir in mammalian NT. This study was conducted to determine the distribution of SP-ir in tiger salamander nasal cavity and forebrain following removal of trigeminal ganglion or section of olfactory nerve (ONx). In control tissue, many SP-ir fibers exist in peripheral olfactory, trigeminal and autonomic nerves, palatine ganglion and outer layers of olfactory bulb. SP-ir bipolar cells, also immunoreactive for gonadotropin-releasing hormone (GnRH), are present along peripheral and central paths of NT. Unilateral removal of the trigeminal ganglion (TRIGx) results in a depletion of, but not total elimination of, SP-ir fibers in olfactory nerve and palatine ganglion. Double-labeled GnRH/SP-ir neurons related in NT are present following TRIGx. Onx does not affect the appearance of SP-ir fibers in any olfactory regions. In addition, GnRH-ir/SP-ir neurons and fibers remain on peripheral and central sides of the transection, although these processes demonstrate swelling near the lesion. GnRH-ir neurons in the preoptic area do not appear to contain SP-ir. This may signify a difference between preoptic and NT GnRH neurons. Supported by NSF grant # IBN 9896098 and University of Oklahoma Undergraduate Research Opportunities Program Grant.

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DO KAINATE RECEPTORS MEDIATE PRE- AND POSTSYNAPTIC EFFECTS?

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Whereas the fast postsynaptic component of excitatory transmission in the olfactory bulb (OB) is likely mostly AMPA receptor-mediated, the role of kainate receptors is unclear. Kainate receptors are expressed by both mitral/tufted (M/T) cells and interneurons, as shown by immunocytochemical labeling in both culture and brain slices. The receptors appear to have a subcellular compartmentalization. In culture and brain slices, kainate receptors occasionally co-localize with synapsin in M/T cell and interneuron dendrites. These results suggest that kainate receptors may participate in dendro-dendritic synaptic transmission. Voltage-clamp analyses indicate that both M/T cells and interneurons express functional kainate receptors and that the ratio of kainate receptor-mediated currents to AMPA receptor-mediated currents is greater in interneurons. These results suggest that the fast postsynaptic component of excitatory transmission may involve both kainate and AMPA receptors. Since kainate receptors appear to be expressed on M/T-cell secondary dendrites, OB kainate receptors also may have a presynaptic role. At a low concentration (1 micromolar), kainate increased excitatory synaptic activity in interneurons-opposite to the effect reported for hippocampal neurons. This effect may be presynaptic, as it was not associated with a postsynaptic current or a change in input resistance. Together, these data suggest that kainate receptors may contribute to both postsynaptic excitatory potentials and presynaptic modulation of neurotransmission. Support:NIDCD and NIH Joint Neuroscience Predoctoral Training Grant.

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EVIDENCE OF PRESYNAPTIC ACETYLCHOLINE RECEPTORS ON ZEBRAFISH OLFACTORY SENSORY NEURONS

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Cholinergic input is thought to modulate neurotransmission, learning, memory and plasticity. We used activity-dependent labeling techniques to explore cholinergic regulation of glutamatergic neurotransmission in the zebrafish olfactory bulb. Acutely dissected olfactory bulbs were incubated in artificial cerebrospinal fluid (ACSF) with tetrodotoxin (1 mM), agmatine: a non-selective cation channel permeant probe (AGB;5mM) and either acetylcholine (Ach, 200μM), Ach + ionotropic glutamate receptor (IGR) antagonists, APV (100μM) and CNQX (50μM), or Ach in Ca^{2+} free ACSF. When stimulated by Ach, a few glomeruli located bilaterally in the ventroposterior region and mitral cells scattered throughout the bulb were AGB immunoreactive. Mitral cell but not glomerular AGB immunoreactivity was abolished in Ca^{2+} free ACSF (to eliminate neurotransmitter release) and by IGR antagonists. Zebrafish mitral cells are thought to receive glutamatergic innervation only from olfactory sensory neurons (OSNs). Therefore Ach is likely activating presynaptic Ca^{2+} (and AGB) permeable nicotinic AchRs on OSNs which results in presynaptic terminal labeling and increases OSN glutamate release. AGB permeation through postsynaptic IGRs accounts for the blockable mitral cell labeling. These results provide evidence that olfactory sensory input may be modulated at the bulbar level by acetylcholine in the zebrafish olfactory bulb. Funded by NIH grants DC01418 and NS-07938.

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LIF mRNA UPREGULATION IN MACROPHAGES AND OLFACTORY RECEPTOR NEURONS FOLLOWING TARGET ABLATION

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Transient upregulation of the leukemia inhibitory factor (LIF) receptor on globose basal cells (GBCs) and ensheathing cells occurs on day (d) 3 post-OBX, coincident with peak GBC proliferation and macrophage infiltration (AChemS XXII, 2000). Now we describe cellular sources of LIF following unilateral OBX using non-isotopic *in situ* hybridization (ISH) with tyramide amplification, and temporal patterns of LIF and interleukin (IL)-6 mRNA expression following bilateral OBX using relative quantitative RT-PCR. Digoxigenin-labeled LIF and olfactory marker protein (OMP) ISH probes were transcribed from SP6-ligated RT-PCR products. At 3d post-OBX, many LIF $mRNA^+$ macrophages infiltrated the OE and ON of the OBX but not control side, and the LIF mRNA signal was upregulated in somas of olfactory receptor neurons (ORNs) on the OBX side. OMP^+ ORNs and $F4/80^+$ macrophages were identified immunocytochemically on adjacent sections; few macrophages were noted on the control side. Relative quantitative RT-PCR demonstrated that mRNAs for LIF and IL-6 were of extremely low abundance in control olfactory mucosa. Following bilateral OBX, mRNAs for both were upregulated as early as 16 hrs post-OBX, with peak increases of 130% for LIF mRNA and 500% for IL-6 mRNA at 3d post-OBX. Our data support the hypothesis that LIF and IL-6, members of the IL-6 family of cytokines, are physiologically relevant factors in intercellular signaling that regulates proliferation leading to neurogenesis in the OE and regeneration of the ON following target ablation. Support: NIH-NIA R01 AG-16824-20 (TVG).

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DOES INTRANASAL APPLICATION OF ZINC SULFATE PRODUCE ANOSMIA IN MICE?

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Recently Slotnick et al. (Behav. Neurosci. 2000, 114:814) reported that intranasal application of ZnSO4 in rats neither destroys the entire olfactory epithelium nor eliminates the sense of smell. To assess this in the mouse, we used the Slotnick et al. operant conditioning and olfactometric procedures to train Swiss Webster derived mice to detect different concentrations (5% - 0.01%) of ethyl acetate and discriminate between 2 complex odors. Mice were treated with .05 ml of 5% ZnSO4 in each nostril (92% survival). Controls were treated with saline. In post-treatment tests, control mice performed at high levels of accuracy on each task but treated mice performed at chance for 5-30 days and then rapidly recovered ability to both detect and discriminate odors. Examination of anterograde transported HRP from epithelium to olfactory bulb in these and additional mice revealed essentially no bulbar input for at least the first 5 days after ZnSO4 treatment. Thereafter, anterograde reaction product was first seen in rostral bulbar glomeruli and, in longer survival mice, was more widely distributed. We conclude that, in the mouse, intranasal application of .05 ml of 5% zinc sulfate produces a frank but short-lived anosmia. Application of 0.1 ml of 5% ZnSO4 produced a longer lasting anosmia but a significantly higher mortality rate.

RETINOIC ACID ENHANCES THE RATE OF RECOVERY OF OLFACTORY FUNCTION FOLLOWING NERVE TRANSECTION

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Retinoic acid (RA) plays an important role in the development of the fetal olfactory system. We recently demonstrated a role for RA in the adult olfactory system. A single administration of RA given one day after bilateral olfactory nerve transection (BNX) enhanced the rate of olfactory recovery in adult, male CBA/J mice[1]. In this study, we evaluated the robustness of this effect in a second strain of mice and examined whether the effect is dose-dependent. Adult male Swiss Webster mice were placed on restricted feeding schedules and trained in a buried food finding task. After 5 days of training (average food finding time ≤ 30 seconds), all the mice received the BNX procedure. One day after surgery, BNX mice that could not find the buried food were then given a single oral dose of all-trans RA (75mg/kg, n=10; 38 mg/kg, n=12; 19 mg/kg, n=11) mixed with 0.1 ml sesame oil or sesame oil alone (oil, n=10). The mice were then tested daily for 25 post-surgical days. Results confirmed our initial finding and showed that the effect of RA is dose-dependent. RA75 group recovered the fastest (defined as three consecutive days of <180 seconds) at post-surgical day 11.3 ± 1.93 followed by RA38 group at post-surgical day 13.6 ± 1.46. RA19 group did not reach criterion until post-surgical day 17.64 ± 2.02 which was similar to OIL group (post-surgical day 16.0 ± 1.03). With time, olfactory performance of all the BNX mice returned to pre-surgical levels. Supported by NIH grants DC004645 and DC00214. 1. Yee, K.K., Rawson, N.E. (2000) Dev Brain Res, 124: 129-132.

ENHANCED ORN EXPRESSION OF HSP25 FOLLOWING OLFACTORY BULBECTOMY

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HSP25 is a member of the small heat shock protein family of molecular chaperones (sHSPs); its expression protects cells against cell death induced by a variety of conditions. The olfactory epithelium (OE) of normal untreated rats shows strong HSP25 immunoreactivity (IR) in basal cells throughout the nasal cavity. However, following exposure of rats to a variety of odorants, strong HSP25 IR also occurs in OE supporting cells (in press). Few or no olfactory receptor neurons (ORNs) show HSP25 IR in either control or odorant-exposed OE. In striking contrast, we noticed that following unilateral olfactory bulbectomy (OB-X) strong HSP25 IR occurs in many ORNs in bulbectomized OE. We have now quantified these observations in 3 day to 6 mos post-OB-X rats. HSP25(+)ORN numbers were often 4-10-fold greater in OB-X than control OE. The largest increases occur at 2 wks post-OB-X, a time of heightened post-OB-X ORN turnover (Exp. Neurol. 115:55, 1992). In contrast, previous findings showed that ORN IR for the 70kD HSP (JCN 348:150,1994) decline in number during the first two weeks following OB-X, mirroring massive ORN loss in this period, but increase by 6 mos post-OB-X (unpublished data). HSP70(+)ORNs also maintain a restricted nasal distribution in contrast to a wider HSP25(+)ORN distribution. These preliminary data suggest that following OB-X 1) ORNs express HSP25 in response to absence of trophic support and 2) HSP25 and HSP70 expressions are independently regulated. Supported by NIDCD grants DC02774 and DC02126.

PERIPHERAL DEAFFERENTATION CAUSES APOPTOSIS IN THE OLFACTORY BULB OF ADULT ZEBRAFISH

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The hypothesis of the current study is that cells in the olfactory bulb are programmed to die following removal of their afferent input from the olfactory organ. Adult zebrafish were anesthetized, and the right olfactory organ was permanently ablated. Programmed cell death was detected in brain sections from fish surviving 1, 6, 12, or 24 hours and 1-3 weeks using Apoptag Red In Situ Cell Death Detection Kit (Intergen), which utilizes the TUNEL method of labeling apoptotic nuclei with a rhodamine tag. Sections were then double-labeled with the Hu antibody (Molecular Probes) as a neuronal marker. Very little apoptotic cell death occurs in normal, sham-operated, and internal control olfactory bulbs. At 1 hour post-deafferentation, there is a significant increase in apoptotic profiles, with most dying cells residing in the olfactory nerve and glomerular layers. This first wave of cell death appears to involve primarily non-neuronal elements. By 24 hours after the surgery, there is another significant increase in cell death. This second wave of cell death appears to include many interneurons of the internal cell layer. The cell death response to removal of afferent input is concluded within the first few days following the surgery, since the number of apoptotic profiles returns to normal levels by 1 week following deafferentation. This study attempts to begin analyzing the mechanisms underlying the interactions between the olfactory organ and bulb, with the long-term goal of elucidating cellular and molecular factors involved in the stabilization of adult brain organization.

OLFACTION IN OLFACTORY BULBECTOMIZED RATS

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Following neonatal olfactory bulbectomy, the cerebral hemisphere grows forward to fill the vacated site and axons of maturing sensory neurons terminate on the surface of or into the paranchyma of frontal cortex. Rats that had been unilaterally bulbectomized on PN2 were trained in an olfactometer at PN80 to detect a variety of odors. The remaining olfactory bulb was then removed and rats were tested on odor detection tasks. Of 20 experimental animals, six retained the ability to detect some but not all odors with at least 85% accuracy. Examination of anterograde transport of HRP*WGA applied to the olfactory epithelium revealed variable numbers of projecting axons and glomerular-like clusters within the anterior olfactory nucleus, frontal pole cortex and piriform cortex in different cases. Factors that may play a role in olfactory detection in animals with direct epithelial-cortical connections are discussed.

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NARIS OCLUSION CAUSES ENHANCED OLFACTORY MARKER PROTEIN IMMUNOREACTIVITY: THE EFFECT OF AGE AT OCLUSION AND LENGTH OF DEPRIVATION

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Olfactory Marker Protein (OMP) is a cytosolic protein of unknown function expressed in mature olfactory receptor neurons (ORN). Recently, olfactory bulbectomy and naris occlusion (NO) were shown to enhance OMP immunoreactivity (IR), results relevant to theories of OMP function including a suggested role in neurogenesis or sensory transduction. Bulbectomy increases while NO decreases neurogenesis in the olfactory mucosa. Therefore, it is unlikely that OMP controls neurogenesis since it is enhanced after treatments that have opposite effects on this process. These results are more consistent with OMP involvement in transduction, perhaps modifying ORN gain in response to odor exposure. To further explore our initial observation we studied the developmental and temporal parameters of OMP enhancement following NO. Mice underwent unilateral NO either one day after birth or as adults. After six days to six weeks subjects were prepared for OMP immunocytochemistry using standard methods. Alternate sections were stained for Neuron Specific Enolase or Phosphodiesterase 4A, the later of which is known to co-localize with OMP. Results suggest that enhanced IR accompanying NO is specific to OMP but is not limited to the developmental period. Preliminary results comparing odor responses in the open and occluded side of the nasal cavity using the electroolfactogram will also be reported.

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A BILE ACID FUNCTIONS AS A MALE SEX PHEROMONE IN THE SEA LAMPREY (PETROMYZON MARINUS)

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Evidence to date support the hypothesis that sex hormones (or metabolites thereof) function as sex pheromones in fish. The sea lamprey, Petromyzon marinus, an ancestral jawless fish, offers a useful model for further testing of this hypothesis. Previous studies suggest that mature male lampreys, which initiate nest construction in rapids of spawning streams, release a pheromone that attracts mature females. It seems likely that this pheromone would have to be produced in amounts larger than sex hormones in order to be detected some distance downstream. Our behavioral assays in both laboratory and field conditions suggest that spermiating males release a potent odorant that induces searching and preference behaviors in ovulated females. This odorant has been identified, by activity directed fractionation and analyses with HPLC, MS, NMR, FTIR and TLC to be a bile acid. This pheromone is released only by spermiating males, attracts ovulated females at sub-nanomolar levels, and represents an alternative to the current leading hypothesis that only excreted sex hormones function as sex pheromones. It is also the first fish pheromone identified through activity directed fractionation. Supported by the Great Lakes Fishery Commission.

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MALE AXILLARY EXTRACTS EFFECT LUTENIZING HORMONE (LH) PULSING IN FEMALE RECIPIENTS

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The human axillae and its secretions appear to be a source of human primer pheromones. Extracts and swabs of both male and female axillary constituents alter the length and timing of the menstrual cycle. These chemical signals could alter reproductive cycle timing by changing the pulse timing of gonadotropin releasing hormone (GnRH). Alterations in GnRH output can be monitored by measuring LH pulsing in blood. Changes in pulse frequency, height and interval occur across a normal menstrual cycle. We hypothesized that a male's primer pheromones could act to alter the menstrual cycle by altering LH pulse parameters and that these changes would best be seen in the follicular phase of the menstrual cycle. Female subjects served as their own controls, receiving either male axillary extract in ethanol, or ethanol, each scented with a deodorant fragrance, by application to the nasal region every 2 hr for 6 hr followed by 6 hr of the opposite stimuli. Application of male axillary extracts affected LH pulse timing causing the average time to the next LH pulse to be significantly shorter (latency after extract 46.45±18.64 min vs.after control 56.03±21.13 min; $p < 0.01$). The results suggest a possible mechanism by which male constituents may alter cycle length. Further, this change in LH can be monitored and used as a bioassay to isolate the physiologically active components of axillary extracts.

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PHEROMONES IN URINE OF RUTTING MALE MOOSE

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Olfactory communication and associated scent-marking activities play a major role in the behavioral ecology of many mammals. During the mating season, scent-marking associated with urine of male cervids is an important chemical cue to relay information to conspecifics. Specifically, adult male moose (Alces alces) dig rutting pits in which they urinate, and females respond to urine deposited in pits. The chemical composition of urine from adult male moose during rut is markedly different when compared with nonrutting adults. During rut, adult males can lose from 12 to 19% of their pre-rut body weight. The catabolism of endogenous reserves as a result of decreased food intake coupled with changes in androgens probably account for the unique smell of urine during rut. We tested the hypothesis that female moose spent more time sampling urine of adult male moose during rut than from nonrut. Urine samples of both nonrut and rut urine were presented to females. Females spent significantly more time smelling samples of rut versus nonrut urine. Only the rut urine elicited the characteristic behaviors associated with the mating season. These results indicate that male urine contains constituents that may act as pheromones which synchronize and prime estrus.

THE SPATIAL ORGANIZATION OF THE PERIPHERAL OLFACTORY ORGAN IN THE ROUND GOBY (NEOGOBIUS MELANOSTOMUS), A TELEOST FISH

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The exotic round goby (*Neogobius melanostomus*) has established populations in all of the Laurentian Great Lakes following initial colonization about ten years ago. Two factors may contribute to the reproductive success of this species. First, it has the ability to spawn repeatedly from spring to fall, and second, males contribute to the success of offspring by guarding and maintaining nests. Both aspects of reproductive behaviour may be mediated by pheromones. A recent study has shown that electro-olfactogram responses and gill ventilation rates increase when the round gobies are subjected to the putative pheromones estrone, 17β-estradiol-3β-glucuronide, and etiocholanolone (Murphy *et al.* 2001. *J. Chem. Ecol., in press*). The present study extends the study of the goby peripheral olfactory organ to examine its spatial organization. Olfactory receptor neurons were visualized in cryostat sections stained for acetylated tubulin immunocytochemistry and by transmission electron microscopy. A continuous sheet of olfactory epithelium was located on a flat surface that extended ventrocaudally from the tentacular anterior nostril. Numerous nonmyelinated nerve fascicles formed in the lamina propria and converged into a prominent olfactory nerve. A sac-like enclosure surrounded by muscular tissue may regulate water flow through the nares. Further research will investigate the relationship between reproductive behaviour and neural activity in the peripheral olfactory organ.

PREFERENTIAL ACTIVATION OF NEURONS IN ROSTRAL ACCESSORY OLFACTORY BULB (AOB) OF MALE HAMSTERS BY FEMALE ODORS

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Previous studies have suggested projection of two vomeronasal organ (VNO) receptor types to different rostral or caudal portions of the accessory olfactory bulb (AOB). In house mice, studies have suggested that odors from females may be selectively processed in the rostral AOB of males. We evaluated the hypothesis that sexual signals from females may be selectively processed in the rostral AOB of male golden hamsters (Mesocricetus auratus). The distribution of Fos-immunoreactive (c-Fos) cells in the AOB of golden hamsters following exposure to conspecific vaginal secretions (CVS) was studied. Following a one-hour exposure to diestrus one CVS, male hamsters were sacrificed and perfused. Tissues were sectioned sagitally and immunocytochemically reacted for expression of c-Fos. Fos-positive cellular counts were recorded and analyzed to assess differing levels of activation among populations of AOB neurons (caudal vs. rostral). We found that there is a significantly greater expression of Fos positive cells in the rostral portion of the AOB. This data will be compated with data on responses to other odors. These results support previous claims of a functional specialization of the rostral and caudal AOB that recieved inputs from different receptor types in the VNO. This dichotomy may represent a peripheral sorting mechanism for sex pheromones or other chemical signals concerning sex and reproduction.

HORMONAL REGULATION OF SYNTHESIS AND DISCHARGE OF A FEMALE NEWT-ATTRACTING PHEROMONE, SODEFRIN

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Sodefrin is a pheromone that specifically attracts sexually developed conspecific female newts, *Cynops pyrrhogaster*. This pheromone is known to be synthesized in the abdominal gland of the male as a form of precursor protein from which an active decapeptide molecule is generated. Treatment of sexually undeveloped newts with prolactin and androgen markedly enhanced the expression of sodefrin precursor mRNA in the abdominal gland and increased immunoassayable sodefrin content in the abdominal gland. The increase of the pheromone (precursor) in the abdominal gland was also confirmed immunohistochemically. It was also found that arginine vasotocin (AVT) decreased the content of sodefrin in the abdominal gland of the sexually developed newts, suggesting that the neurohypophyseal hormone induces the discharge of sodefrin. Administration of a V1 (vasopressor) receptor antagonist, but not a V2 (antidiuretic) receptor antagonist to the courting males blocked the decrease of sodefrin content. Existence of actin-like protein in a structure around the ducts of the abdominal gland suggests that AVT acts on this structure to contract and in consequence induce the discharge of the pheromone.

HUMAN PHEROMONES AND A MAMMALIAN MODEL OF MATE PREFERENCE

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Human pheromones alter luteinizing hormone/follicle stimulating hormone (LH/FSH) ratios. This effect links social-environmental sensory input ("nurture") to the genetically determined embryonic/prenatal development ("nature")of the gonadotropin releasing hormone (GnRH) neuronal system, and to the hypothalamic GnRH pulse. GnRH pulsatility modulates the concurrent maturation of the mammalian neuroendocrine system, the reproductive system, and the central nervous system. This pheromonally influenced, GnRH-directed, mammalian neuroendocrine model of sexual differentiation, sexual development, sexual orientation, and sexual behavior may explain many aspects of human sexuality through its correlative and predictive power. For example, this mammalian model suggests that pheromones and olfaction are more important to the development of human sexuality than are visual stimuli. In addition, genetically determined, sexually differentiated, physically attractive characteristics have hormonal, and presumably pheromonal components. The absence of a mammalian neuroendocrine model for sexual attraction that is primarily based on visual input suggests that olfactory input conditions visual aspects of human sexual attraction. Pheromone production/distribution, olfactory acuity/specificity, and hormone responses elicited by human pheromones may explain the development of human mate preferences, just as they explain mammalian mate choice and properly timed reproductive sexual behavior.

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CONTEXT AFFECTS ANS AND PSYCHOLOGICAL RESPONSES TO PUTATIVE HUMAN PHEROMONES

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We previously observed psychological responses to the human chemosignals Δ4,16-androstadien-3-one and 1,3,5,(10),16-estratetraen-3-ol. We wished to determine whether these compounds have corresponding autonomic nervous system effects and if such effects are modulated by the presence of a man or woman during sessions (from our hypothesis that these steroids modulate responses to the environment rather than releasing specific cognition). We tested men and women in a double-blind, within-subject, repeated measures experiment, half of each sex with a male tester and half with a female tester. In the 20 minutes after test solutions were applied under the nose, women showed clear physiological responses to both compounds, but did so only in the presence of the male tester. Mens' responses were not affected by the sex of the tester. Similarly, women responded psychologically to androstadienone with an increase in positive/stimulated mood only in the presence of a male tester, while men's did not respond to either compound. These data suggest that these steroids interact with social or biological context when producing both psychological and physiological effects. Precisely what aspects of the testers produced this modulation is currently being studied. The mechanism by which these compounds produce these changes is not clear from our study, leaving open the the issue of whether they constitute human pheromones or a class of chemosignal associated with olfactory qualities (*vasanas*). Supported by: Macarthur Foundation, NIH MERIT R37 MH41788 (McClintock) & Tova Fellowship (Jacob).

202 Poster XVII. Sweet Taste

COMPARATIVE TASTE RESPONSES IN VERTEBRATES TO COMPOUNDS SWEET IN HUMANS

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Using two-bottle preference tests combined with behavioral observations, the gustatory responses of various animal species to compounds sweet in humans were analyzed. The first amazing observation was the dichotomous responses of primates to thaumatin and aspartame which separated the order of primates into two groups: (i) Old Worlds monkeys in which they are both effective; (ii) New World monkeys and prosimians in which they are inactive. The comparative study was extended to various sweeteners, natural carbohydrates and artificial sweeteners (e.g. guanidine derivatives like carrelame, bernardame, sucrononate and lugduname, dipeptide derivatives like aspartame and neotame, saccharin, acesulfame-K, Na-cyclamate and sucralose) and to various farm and house animals and fishes, and, more recently a bird, the red-legged honeycreeper (Cyanerpes cyaneus) and a reptile, the gecko (Phelsuma madagascariensis grandis). With these two species, we observed for example that, among the natural carbohydrates tested to date, only fructose, glucose, maltose, sucrose and raffinose elicit a preference in the red-legged honeycreeper (with a lower effectiveness compared to humans) and only fructose, glucose and sucrose in the gecko. Both species do not show any gustatory preference to the most potent sweeteners found in humans (the guanidine sweeteners) or to the other artificial sweeteners tested so far. This research is a part of the EU-project:'The Optimisation of Sweet Taste Quality'and was supported by the BBW, Bern, Switzerland.

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COMPUTATIONAL STUDIES OF SWEET TASTING COMPOUNDS

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We have developed Quantitative Structure Activity Relationships (QSARs) for families of sweet tasting compounds including isovanillates, sulfamates and sucrose derivatives. Multiple regression equations have been obtained via the genetic algorithm using both Physicochemical Descriptors (PD) and interaction energies from Molecular Field Analysis (MFA). 41 isovanillyl derivatives have Relative Sweetness (RS) values spanning from 50 to 20,000. QSARs using MFA descriptors resulted in a best fit (r^2=0.746, r^2_{CV}=0.607), but those from PD also had significant predictive power. QSARs were also developed for 40 halide-substituted sucrose molecules (RS 0.2 to 7,500). Both PD and MFA parameters gave good equations with significant predictive power (r^2=0.883, r^2_{CV}=0.829 and r^2=0.904, r^2_{CV}=0.860 respectively). Pseudo-receptor studies were carried out on these two sets. An artificial receptor was built and optimised using ligand equilibration, in which correlation-coupled receptor optimisation and free ligand relaxation were altered till the optimum correlation is obtained. For the isovanillate derivatives the correlation coefficient for $\Delta G_{PRED}/\Delta G_{CALC}$ was 0.97 indicating good agreement. These computational studies have enabled us to explain the RS values of compounds in terms of molecular descriptors and also to predict the taste of compounds as yet unsynthesised or tested. We thank the EC (FAIR-PL-98-4040) for support.

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TASTE DEFECTS IN DROSOPHILA NORPA MUTANTS

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Phospholipase C (PLC) is a membrane associated enzyme catalyzing hydrolysis of a minor membrane phospholipid, phosphatidylinositol-4,5-bisphosphate (PIP2). Products of this reaction - inositol-1,4,5-trisphosphate (InsP3) and 1,2-diacylglycerol (DAG) - are involved in a number of cellular responses e.g. opening of calcium stores and activation of protein kinase C (PKC). In Drosophila, PLC is encoded by norpA (no receptor potential A) gene expressed in compound eyes, antennae, maxillary palps and labellum. Consistent with this expression pattern NORPA has been found to play a significant role in vision and olfaction but its role in taste perception has not yet been studied. We have investigated gustatory responses in Drosophila norpA mutants using behavioural and electrophysiological approaches combined with pharmacological and genetic analysis. The alleles used included near-null norpA P24, a temperature-sensitive allele, norpA H52, as well as several hypomorphic alleles. These mutants show a various degrees of decrease in sensitivity to sweet compounds (e.g. sucrose) suggesting that the PLC may play a significant role in Drosophila taste perception.

HYDRATION PROPERTIES AND PROTON EXCHANGE IN AQUEOUS SUGAR SOLUTIONS STUDIED BY TIME DOMAIN NUCLEAR MAGNETIC RESONANCE (TD-NMR)

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ABSTRACT NMR relaxation rates (R1 & R2) were measured in aqueous solutions of Sucrose,D-Glucose and D-Fructose with increasing concentration(5 to 35%w/v). The measurements were carried out using Bruker PC 20 NMR Process analyzer. Inversion Recovery and CPMG pulse sequences are used for the measurement of relaxation rates. Results show that the values of relaxation rate increase as the concentration of the sugar is increased. The relaxation rates are found to be higher for D-glucose solutions as compared to Sucrose or D-Fructose solutions. These results were discussed on the basis of molecular association between sugar and water molecules through hydrogen bonding. The dependence of (R2) on the interpulse delay of the CPMG sequence gives information on the proton exchange mechanism involved. The mechanism of exchange was studied using (R2) with increasing inter pulse delay of 0.1 to 9.0 ms in aqueous solutions of 10, 20 and 35 % (w/v) of the above Sugar solutions. From the plots of relaxation rates (R2) versus the 90-180° pulse spacing it was possible to calculate the proton exchange rate (kb) of the different sugar solutions. Relaxation rates show characteristic variations with CPMG pulse spacing which can be interpreted on the basis of chemical exchange between solute and solvent molecules. The experimental results namely relaxation rates and CPMG pulse spacing data show the importance of water interactions with sweet molecules and this can lead to a better understanding of the effect of hydration water in sweet taste chemoreception.

SWEET RECEPTOR MECHANISMS: STIMULATING EFFECTIVENESS OF HIGH INTENSITY SWEETENERS AND ENANTIOMERS FOR FLY BEHAVIORAL AND SUGAR RECEPTOR CELL RESPONSES

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Sweet taste transduction in vertebrates is thought to involve a cAMP pathway for natural sweeteners and an IP3 pathway for high intensity sweeteners. As a first step to investigate these mechanisms in flies, we studied Phormia regina proboscis extension responses (PER) to various sweeteners and also tip-recorded the extracellular responses from single sensilla in isolated proboscis preparations. Stimuli were sucrose, aspartame, sodium saccharin, sodium cyclamate and the halogenated sugar, sucralose, across concentration ranges effective in humans (Schiffman & Gatlin, Neurosci. & Biobehav. Rev. 1993, 313). Flies that gave a PER to sucrose 64mM were tested with each concentration of a high intensity sweetener (9 flies/sweetener), but none gave a PER to other sweeteners. There was a typical concentation-response function for the sugar receptor cell firing to sucrose ($p< 0.003$), but not for firing to other sweeteners ($p>0.05$)(Repeated Measures ANOVAs). These data suggest a high intensity sweetener/IP3 pathway, as proposed for vertebrates, is not excitatory for the fly sugar receptor cell and are consistent with the suggestion of Ozaki et al (J.Gen.Phys. 1992, 867) that IP3 is involved in adaptation of the fly response to sucrose. In ongoing work, the nature of the receptor component of the transduction process is being examined by stimulating with enantiomeric pairs of sweeteners. Preliminary results support a protein receptor-ligand interaction over a purely physicochemical process.

CATION EFFECTS IN CYCLAMATE SWEETENERS

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Sodium and calcium cyclamate are permitted sweeteners in over 55 countries and are considered well-liked, especially when blended with saccharin. Synthesising cyclamate derivatives ($C_6H_{11}NHSO_3^-M^+$) has allowed solution chemistry investigations which may lead the way in optimising sweet taste quality. There are reports that Cs, K and Rb ions are net water structure breakers and that they are associated with bitter perception. To determine taste and solution effects in cyclamates, it is also important to compare ions with similar ionic radii (and therefore volumes) to elucidate the role of charge density. Of the four salts Ba, Cs, Sr and Rb cyclamates, only the latter two have apparent specific volumes (ASV) within the sweetness range (0.51-0.71 cm^3g^{-1}) but interestingly only Cs, and Rb cyclamates tallied in their apparent specific volumes with theoretical calculations based on literature values. These two had high ionic radii and hence low charge densities. They would therefore alter water structure less. Their high apparent specific isentropic compressibilities accord with this interpretation, which has already been employed to explain the sweetness-enhancing properties of magnesium salts. Most cations depress ASV and Cs, Rb and K are probably the only elemental cations which have the opposite effects. The derivatives differ markedly in their solution effects and these may alter their taste modalities.

A RATIONAL DESIGN OF NEW INTENSIVE SWEETENERS FROM NATURAL COMPOUNDS

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The use of natural compounds as leading molecules to develop new molecules is common, but in the case of the sweeteners only few compounds have been actually obtained in this way. Most of the known sweeteners have been instead discovered by chance, or designed from rational modifications of known molecules. Isovanillic derivatives are analogues of the natural dihydroisocoumarin R-(+)-phyllodulcin. Our group extensively studied the structure-activity relationships of this class of compounds. [3, 4]. Either the primary structure, or the conformation and the configuration proved to be important in taste. The isovanillic fragment seems to correspond to the AH-B glucophores of Shallenberger and Acree [5] and is essential for the sweet taste. We have prepared some new compounds by attaching an hydrophobic fragment to different polar/hydrogen bonding blocks taken from molecules of different classes. The hydrophobic fragment is either aromatic, aliphatic or heterocyclic and comes from isovanillic compounds, aspartame, or terpenes; the polar block is chosen among peptides or peptidomimetics, nucleic bases and the isovanillic ring. [1] Asahina, Y.and Asano, J.(1929) Chem. Ber., 62: 171. [2] Vleggaar, J. Ackerman, L.G.J. and Steyn, P.S. (1992) J.Chem. Soc. Perkin Trans. 1, 3095. [3] Arnoldi, A., Bassoli, A., Borgonovo, G., Drew, M.G.B., Merlini, L. and Morini, G. (1998) J. Agric. Food Chem., 46: 4002. [4] Bassoli, A., Merlini, L., Morini G. and Vedani, A. (1998) J. Chem. Soc. Perkin Trans. 2,, 1449. [5] Shallenberger R.S. and Acree, T.E. (1967), Nature, 216, 480.

NEUROPHYSIOLOGICAL TASTE RESPONSES OF SINGLE GENICULATE GANGLION CELLS RECORDED *IN VIVO* IN THE RAT

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There is a paucity of information regarding neurophysiological taste responses from neurons that innervate palatal receptors and from neurons contained within the geniculate ganglion. To address these deficiencies, the present study characterized the response properties of greater superficial petrosal and chorda tympani neurons by recording *in vivo* from single geniculate ganglion cells. Once a taste-responsive neuron was found, solutions of 0.1 and 0.5 M NaCl and NH_4Cl, 0.5 M sucrose, 0.01 N HCl and 0.01 M quinine were applied to the whole mouth. The palate and tongue were then isolated to determine the receptor population of the neuron. In addition, after taste profiling, a neuronal tracer was injected into the cell soma to determine the morphology of the neuron's terminal in the nucleus of the solitary tract. Responses from chorda tympani neurons were similar to that previously noted in single-fiber studies; they responded strongly to NaCl and NH_4Cl stimuli and were generally less responsive to sucrose, HCl and quinine. Greater superficial petrosal neurons also responded well to NaCl and NH_4Cl. The difference in the two populations was most notable for sucrose; a small percentage of greater superficial petrosal neurons responded vigorously to sucrose. These sucrose response frequencies far exceeded responses elicited by any other stimulus in either population of neurons. Results suggest that the strong whole-nerve response of the greater superficial petrosal nerve to sucrose may be the result of the activity of a small subset of neurons. NIDCD: DC03500 (SIS), DC00407 and DC03576 (DLH).

CALCIUM DEPRIVATION ALTERS GUSTATORY-EVOKED ACTIVITY IN THE RAT NUCLEUS OF THE SOLITARY TRACT

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Calcium-deprived rats develop a compensatory appetite for substances that contain calcium. This appetite is expressed in short-term tests and under sham-drinking conditions, when primarily gustatory factors are involved. In addition, whole-nerve chorda tympani responses to the taste of $CaCl_2$ are altered by calcium deprivation. To further investigate the role of gustatory factors in calcium appetite, we recorded the extracellular activity of single neurons in the nucleus of the solitary tract (NST) of calcium-deprived and replete rats. Calcium deprivation was induced by maintenance on a low calcium diet (25 mmol/kg calcium carbonate) for 6-11 weeks, which resulted in significantly reduced plasma ionized calcium levels. We recorded the evoked activity of 51 neurons from replete rats and 47 neurons from calcium-deprived rats following application of a broad array of taste stimuli. In a subset of these cells, responses were also recorded to a concentration series of $CaCl_2$ ranging from 0.01 to 300 mM. In analyses across all neurons, there were no differences in responding between the groups. However, NST neurons with sugar-oriented response profiles gave significantly larger responses to $CaCl_2$ in the calcium-deprived group than did corresponding cells in the replete group. This difference in taste-evoked responding may cause an increase in the palatability of $CaCl_2$ and, in turn, contribute to the expression of calcium appetite in rats.

HAMSTER STIMULUS GENERALIZATION PATTERNS FOR TASTE MIXTURES

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Integration of information about mixtures occurs in the gustatory periphery and is evident in suppression of sucrose responses by quinine.HCl (QHCl) and suppression of QHCl responses by NaCl in the hamster (*Mesocricetus auratus*) chorda tympani (CT) (Formaker & Frank (1996) Brain Res). Consequences of these physiological interactions were investigated with a conditioned taste aversion study. Seven experimental groups of 8 hamsters were each trained to avoid one conditional stimulus (CS) by pairing with LiCl injection. Conditional stimuli were 100mM NaCl (N), 100mM sucrose (S), 1mM QHCl (Q), plus the 4 binary-ternary mixtures of those stimuli. The control group (n=16) CS was deionized water. Each animal underwent 2 conditioning trials and 2 cycles of testing with 8 test stimuli, identical to the conditional stimuli. Percent suppression of intake (mL) of each test stimulus (TS) by each animal was calculated relative to mean control TS intake. Patterns of suppression of the 8 test stimuli across the 7 CS groups were established and similarities between the patterns quantified by correlation coefficients. Generalization patterns for the 3 individual components were distinct, but 3 pairs of TS patterns were highly positively correlated: S and the SQ mixture (r= 0.79, p=0.034), N and the NQ mixture (r=0.86, p=0.014), and the SN and SNQ mixtures (r=0.83, p=0.022). Effects of conditioning were retained for months. Overall, aversions to mixtures and mixture components generalized except for quinine, which was not recognized in quinine-NaCl mixtures (Formaker et al. (2000) Chem Senses). Supported by NIH DC04099.

MULTIMODAL MONOSODIUM L-GLUTAMATE (MSG) SENSITIVITY IN HUMAN

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NaCl and MSG thresholds were repeatedly measured in 171 subjects using a paired comparison test associated to the Up and Down procedure[1]. Suprathreshold sensitivity was evaluated, in 119 subjects, as the concentration of MSG eliciting the same intensity as a 29mM NaCl reference solution. None of the distributions fitted the normal law ($p<0.001;\chi^2$). A multigaussian analysis could split the MSG threshold distribution into several subpopulations. 73% of the 109 subjects common to both experiments presented a significantly different sensitivity to MSG compared to NaCl on a molar basis (Student t), whereas 27% presented a non significantly different sensitivity to both stimuli and could be classified as glutamate more or less severe hypotasters. A qualitative discrimination task was presented to 8 hypotasters including 24 triangular presentations of NaCl/MSG 29mM per subject. Two of them could not discriminate between both stimuli ($p<5\%$, α error) indicating they probably perceived only the sodium cation in the 29mM MSG solution. The remaining six hypotasters could discriminate MSG from NaCl, although they were not able to either identify any specific taste in 29mM MSG. PTU and MSG hypogueusiae were shown independent. Time intensity profiles were significantly different for normo- and hypotasters. MSG hypogueusia points to multiple receptors and individual genetic differences of MSG taste sensitivity. [1]DIXON W.J., MASSEY F. In: Introduction to statistical analysis, Mac Graw Hill, NY, 19, 377-, 1960 Acknowledgements: IGTC, COFAG.

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DYNAMIC AND MULTIMODAL RESPONSES IN RAT GUSTATORY CORTICAL SINGLE NEURON ENSEMBLES

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Subsets of the neurons in gustatory cortex (GC) have been identified as taste-specific on the basis of overall firing rates. Little is known about the time course of GC responses in awake animals, although evidence from many sources suggests that such responses should be modulated through time. We delivered multiple trials of an array of tastants to awake restrained rats while examining the temporal response properties of GC single-neuron ensembles. Consistent with earlier studies, 14% of the GC neurons were taste-specific according to overall firing rates. The number climbed to 40% when time was taken into account, however, because many neurons produced phasic or biphasic responses. There appeared to be 3 primary sources of response modulation—an early somatosensory source related to the stimuli striking the tongue, a slower source related to chemosensory processing, and a late source related to palatability of the tastes. This late source also appeared to be largely somatosensory, and may reflect the onset of taste-related orofacial behaviors. The middle, chemosensory component of GC responses was itself time-varying, perhaps due to the time course of peripheral responses, or to between-neuron interactions, or both. Our evidence suggests that GC neuronal pairs do interact in tastant-specific ways, even when one or both of the neurons in the pair were not themselves taste-specific according to any firing rate analysis. Overall, these data suggest that gustation may be broader, more distributed, and more a result of interaction between neurons than has been previously thought.

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CROSS ADAPTATION AND BITTER SUPPRESSION OF L-TRYPTOPHAN AND L-PHENYLALANINE: FURTHER SUPPORT FOR COMMON TRANSDUCTION MECHANISM

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Recent research in our lab (Delwiche et al., Perception and Psychology, 2001, In Press) has shown that bitter compounds may cluster together as a function of individual sensitivities. One tight cluster was L-tryptophan (trp), L-phenylalanine (phe) and urea. The present research assessed if trp, phe, urea, and quinine cross adapt each other. Also, whether the bitter taste of these compounds and magnesium sulfate is reduced by addition of sodium salts. Subjects (n=13) intensity matched bitter compounds using the Labeled Magnitude Scale. Adaptation experiments reveal trp cross-adapted phe 84%± 4, and phe cross-adapted trp 91%±3.3, but the amino acids were not as effective at cross-adapting urea (trp 55%±9.6, phe 71%±5.9) or quinine (trp 49±7.2, phe 48±6.8). Urea cross-adapted bitterness of the amino acids (trp 85%±3.3, phe 84%±4.5), and quinine (69%±6). Quinine-HCl cross-adapted approximately half of the amino acid bitterness (trp 46%±9.9, phe 41%±10.2) and approximately quarter the bitterness of urea (27%±3). With the addition of sodium salts, chloride and gluconate, bitterness of both amino acids was suppressed to comparable degree (60%). Quinine and urea bitterness was suppressed to a lesser degree (50% and 40% respectively), and bitterness of magnesium sulfate was not reduced. Results indicate 85-90% of the bitter taste elicited by trp is common to phe and vice versa. Urea bitterness cross adapts 85-90% of elicited amino acid bitterness, but urea appears to have amino acid independent bitter taste mechanisms as cross adaptation by amino acids was between 55-70%.

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TASTE-TASTE AND TASTE-SMELL INTERACTIONS LIMIT THE PERCEPTION OF COMPONENTS AND INPUT TO FLAVOR

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It is difficult for humans to identify more than 3 components in a mixture of odorants (Livermore & Laing, 1996). The present two experiments investigated whether there is a similar limitation in the analysis of taste mixtures and whether the parallel processing of the components of taste-odor mixtures by the gustatory and olfactory systems increases the number of components identified. Subjects were trained to identify the components presented alone and to use a selective attention procedure to identify them in mixtures. All stimuli were presented using a retronasal (by mouth) technique. A maximum of three tastants were identified in both types of mixtures, and no component was identified in four component odor-taste mixtures. Importantly, in no instance was the olfactory stimulus identified in any mixture with tastes. Three mechanisms are proposed to account for the results, one of which is the proposal by Rozin (1982) that the identity of odorants will be lost in mixtures with tastes due to a combining of the two percepts. Implications of these data for the perception of flavor will be discussed. Livermore, B.A. & Laing, D.G. (1996). J.Exptl. Psychol. 22,267-277. Rozin, P. (1982). Percept.Psychophys. 31, 397-401.

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SIGNAL TRANSDUCTION IN THE VOMERONASAL EPITHELIUM OF MAMMALS AND REPTILES

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There is now considerable evidence from studies in mammals and reptiles that the signal transduction mechanism in the vomeronasal (VN) system involves ligand binding to G-protein coupled receptors, an increase in 1,4,5-inositol trisphosphate (IP3) and subsequent increases in intracellular calcium. In most cases, there is also a decrease in cAMP and several components of the cAMP pathway are present in the VN epithelium, yet the role of this pathway in VN signal transduction is poorly understood. At least two populations of receptor cells expressing members of different families of G-protein coupled receptors appear to be involved in differential response to VN stimulants in mammals. The increase in IP3 resulting from stimulation with different VN stimulants is not uniform in these two populations of cells. Different subsets of cells respond differently depending on the source or nature of the stimulating substance.

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MOLECULAR MECHANISMS OF PHEROMONE DETECTION IN MAMMALS

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In order to characterize new components of the mammalian vomeronasal signaling pathway, we have undertaken a systematic comparison of transcripts expressed by individual MOE and VNO neurons. Recently we identified a novel multigene family that encodes novel seven-transmembrane domain receptors, and is likely to represent a new class of pheromone receptors in mammals, the V3Rs. Our data suggest that the V3R family comprises over 100 genes. The expression of V3Rs is strictly restricted to the VNO, and the V3Rs are found in the apical portion of the neuropithelium in a specific subpopulation of sensory neurons that lack expression of both V1Rs and V2Rs, the two other families of candidate pheromone receptors. The V3R family is distantly related to two other families of mammalian chemosensory receptors, the putative pheromone receptors V1Rs, and the taste receptors T2Rs. The identification of the V3R gene family reveals an unexpected molecular and cellular complexity in VNO function: from our study the VNO emerges as a composite sensory organ, in which at least 3 distinct populations of chemosensory neurons, the V1R-, the V2R- and the V3R- expressing neurons, are intermingled. This organization sharply contrasts with the relatively simpler cellular and molecular structure of the main olfactory epithelium in which a unique type of olfactory receptor has been identified. The elucidation of the functions for the different classes of candidate pheromone receptors is likely to provide significant insight into the coding of pheromone signals that govern specific behaviors.

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PHEROMONE TRANSDUCTION BY MOUSE VOMERONASAL NEURONS

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We have developed a comprehensive approach by which the mechanisms underlying transduction and adaptation of chemosignals in sensory neurons of the mouse vomeronasal organ (VNO) can be investigated. This approach is based on the use of eletrophysiological and high resolution confocal Ca2+ imaging techniques in three different preparations: (a) a VNO whole-organ preparation; (b) acute VNO tissue slices; and (c) freshly dissociated vomeronasal neurons (VNs). The first step in this analysis is to derive the receptive field of single VNs which may be equivalent to the receptive field of a given vomeronasal receptor in that neuron. Our results show that several pheromones with known biological functions induce excitatory responses in single VNs leading to action potential generation and elevated Ca2+ entry. VNs can detect these compounds with extreme sensitivity and specificity, which is in contrast to the relatively broad tuning properties of olfactory receptor neurons (ORNs). We have begun to dissect the molecular steps mediating these signal transduction events. The results provide clear evidence that VNs use second messenger mechanisms that are distinct from those in ciliated ORNs. There is increasing evidence that phospholipase C is a key enzyme for pheromone transduction in VNO. Supported by grants from NIDCD, NINDS, and NSF.

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A MEMBER OF THE TRP FAMILY OF ION CHANNELS MAY MEDIATE VNO SENSORY TRANSDUCTION

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In vertebrates, two nasal chemosensory organs allow animals to detect complex chemicals in the environment: the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). Despite similarity in function, recent work has indicated that these two chemosensory systems have evolved distinct sensory transduction cascades. Physiological and molecular experiments have shown that transduction in the VNO is likely to be independent of cyclic nucleotide-gated ion channels, which mediate transduction in the MOE. We hypothesized that, instead, VNO transduction might bear similarity to cyclic-nucleotide independent signaling pathways in invertebrates. Indeed, we identified a channel in the VNO, TRP2, which bears similarity to dTRP, the light-activated channel that mediates phototransduction in *D. melanogaster*. TRP2 is expressed specifically and abundantly by VNO sensory neurons and the TRP2 protein is immunolocalized to sensory microvilli, consistent with a role for TRP2 in VNO sensory transduction. Interestingly, the TRP2 gene is a pseudogene in humans, in agreement with the notion that the human VNO is vestigial. Sequencing of the TRP2 gene in other primates thus presents an opportunity to determine when in evolution this change occurred. Supported by the NIDCD.

220 Poster XXX. Cortical Processing in Chemosensory Systems

REPEATED TRIGEMINAL STIMULI RESULT IN A CONSTANT OR EVEN SENSITIZED SII ACTIVITY WHILE OLFACTORY RELATED ACTIVITY GENERALLY SHOWS ATTENUATION

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Functional magnetic resonance imaging (fMRI) is a non-invasive brain imaging technique. In this study, we used fMRI to investigate the areas of the brain responsible for olfactory processing during repeated presentation of vanillin and tea aroma. 8 subjects participated in the study. T2*-weighted, coronal images were obtained using a 3 T scanner. Odourants were delivered in bursts within an ON period, followed by an OFF period with a constant flow of clean air. This procedure was then repeated for 40 cycles, alternating between the tea and vanillin odour using an olfactometer. Some subjects indicated that the stimulation caused a painful (i.e. trigeminal) sensation. Activated areas were identified via correlation analysis. Significant areas of activation were found bilaterally in the inferior frontal gyrus, in the left superior temporal gyrus, left orbitofrontal cortex, cingulate gyrus, right SII cortex, and bilateral pre-motor areas. In the majority of brain areas, the measured activation showed a significant attenuation over the 40 cycles of the experiment, consistent with effect of habituation. However, activation in SII was found to be relatively constant over the duration of the experiment indicating a trigeminal response.

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OLFACTORY EVENT-RELATED POTENTIALS TO ORTHO- AND RETRONASAL STIMULATION

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The present study aimed to investigate differences between ortho- and retronasal perception using olfactory event-related potentials (OERP). A total of 9 subjects (4 f, 5 m, 19–45 years) participated. Orthonasal olfactory function was tested using "Sniffin`sticks", retronasal olfactory function was assessed using an identification test consisting of 20 aromas applied orally. OERP were recorded to ortho- and retronasal stimulation. For retronasal stimulation odorants were applied via tubing placed below the lower turbinate. For stimulation phenylethylethanol (PEA) and H2S were used. OERP to ortho- and retronasal stimulation showed good correlation (e.g. ampl. N1P3, 2 ppm H2S – r=.92, 8 ppm - r6=.88). OERP to ortho- and retronasal stimulation discriminated between stimulus intensities. Significant effects in relation to the site of stimulation were found for N1 latency, which was shorter after orthonasal stimulation. These results indicate, that OERP are suitable for the investigation of cortical information processing in relation to ortho- and retronasal stimulation. Responses to ortho- and retronasal stimulation appeared to be similar in many respects. However, the observed differences for N1 latencies indicate different mechanisms of information processing depending on the site of olfactory stimulation.

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INSULAR NEURONS ENCODE SALIENCE OF TASTE CUES IN AN ORAL DUAL-REINFORCER SELF-ADMINISTRATION PARADIGM

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Multiple studies have suggested that the insular cortex is not involved in fundamental taste identification, but rather in higher cognitive processes influencing ingestive behavior. The goal of this study was to characterize response profiles of insular neurons across a paradigm where taste cues are the discriminative information modifying oral self-administration behavior. Male Long Evans rats were trained to orally self-administer a liquid reinforcer. Electrodes were then chronically implanted in the insular cortex. Neural activity was recorded across a paradigm where animals were required to nosepoke on a FR1 schedule. A 0.5 to 1.5 sec variable delay, a 0.5 sec tone, and a 100 µl drop of one of two liquid reinforcers into a single spout followed the nosepoke. Because reinforcer availability was presented in fixed alternating timing loops, the taste of a reinforcer predicts its future availability. A subset of insular neurons displayed phasic neural firing patterns timelocked to the consumption of the reinforcer. These patterns were neither static nor linked to the identity of the reinforcer, but reflected self-administration behavior patterns. Activity of single neurons could be modified by contrast of hedonic value within reinforcer pairs, altered interoceptive state, or with experience. These data suggest that the insular cortex is involved in assessing the contextual relevance of taste cues as they contribute to an ingestive behavioral strategy.

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ENHANCED CHOLINERGIC SUPPRESSION OF PREVIOUSLY STRENGTHENED SYNAPSES ENABLES THE FORMATION OF SELF-ORGANIZED REPRESENTATIONS IN OLFACTORY CORTEX

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Many abstract models of associative memory function ignore the influence of changes in synaptic strength during the storage process and apply these changes only during a so-called recall-phase. Efforts to ensure stable activity with more realistic, continuos updating of the synaptic strength during the storage process have shown that the memory capacity of a realistic cortical network can be greatly enhanced if cholinergic modulation suppresses the synaptic weights of the association fibers during the learning process. We here present experimental data from an olfactory cortex brain slice preparation showing that previously potentiated fibers are significantly more suppressed by the cholinergic agonist carbachol than unpotentiated fibers. We conclude that low suppression of non potentiated fibers during the learning process ensures the formation of self-organized representations in the neural network while the higher suppression of previously potentiated fibers minimizes interference between overlapping patterns. We show in a computational model of olfactory cortex, that, together, these two phenomena reduce the overlap between patterns that are stored within the same neural network structure.

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THE ROLE OF ACETYLCHOLINE IN ODOR DISCRIMINATION AND CROSS-HABITUATION BY ANTERIOR PIRIFORM CORTEX NEURONS

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The mammalian olfactory system is heavily innervated by cholinergic inputs from the horizontal limb of the diagonal band. These cholinergic inputs have modulatory effects on several olfactory functions including: olfactory memory, signal to noise characteristics, and information throughput and coherence between the different regions of the olfactory pathway (Chabaud et al, 2000; Hasselmo, 1995; Ravel et al 1994). We have recently demonstrated that odor discrimination is substantially better in the anterior piriform cortex (aPCX) than in the main olfactory bulb (Wilson, 2000). The present study examined the role of aPCX ACh muscarinic receptors in this heightened odor discrimination. Adult Long-Evans rats, urethane anesthetized and freely breathing were used as subjects. Odor receptive fields of aPCX single-units to alkane odors were mapped before and after either a systemic injection of the muscarinic receptor antagonist scopolamine (0.5mg/kg) or aPCX surface application of 500 µM scopolamine (or saline/ACSF controls). Cross-habituation between alkanes differing by 2-4 carbons was then examined following a 50 sec habituating stimulus. The results demonstrate that cross-habituation in aPCX neurons was significantly enhanced (odor discrimination decreased) by either systemic or cortical scopolamine. Given that ACh primarily effects intra-cortical association fibers in the aPCX (Hasselmo & Bower, 1992), the results support a role for the association system in odor discrimination and suggest an important ACh modulatory control over this basic sensory process. Supported by NIDCD.

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THE INFLUENCE OF CONTEXT ON PRIMARY OLFACTORY PROCESSING

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The principal neurons (mitral cells; MC) in the mammalian olfactory bulb (OB) relay sensory input to other brain areas. MCs receive direct and indirect sensory input, and much is known about the mapping of input fiber projections onto them. In anesthetized animals, the spatial organization of MC responses suggests chemotopy. Few studies have recorded MC activity in a strictly timed behavioral paradigm. We present evidence that MC activity during and around behavioral odor identification is shaped largely by what the stimulus predicts to the animal. Rats were implanted with six moveable microelectrodes, positioned over the dorsomedial OB, and trained in a simple odor-cued taste-avoidance task. This enabled us to isolate the initial odor stimulus to a 0.5 - 1.0 sec. window. Of the 52 MCs unambiguously identified only 11% showed odor-selectivity, while 94% showed task-specific firing rate modulation. These patterns depended reversibly on the meaning of the stimulus, and odor-selectivity was altered when the meaning of the stimulus changed. The OB receives substantial synaptic and neuromodulatory input from other brain areas. Simultaneous local field potential (LFP) recordings in the OB, prepyriform cortex (PPC), entorhinal cortex (EC), and dentate gyrus address this issue. Profound changes in the frequency structure of the LFP in the OB and PPC during odor identification are preceded by input from the EC. Thus, centrifugal input to the OB may influence MC responses during behaviorally significant odor exposure. *Supported by NIDCD, Burroughs-Wellcome and Keck Foundations..*

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INTRA-CLASS CORRELATION COEFFICIENTS FOR BETWEEN-SESSION MEASUREMENTS OF THE OLFACTORY EVENT-RELATED POTENTIAL

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Olfactory event-related potentials (OERP) have been studied in the investigation of olfactory processing in health and disease. However, the reliability of this measurement has yet to be established statistically. The present study examined the test-retest reliability of the OERP in young and older adults with a between-session interval of 4 weeks. In each session, a single-stimulus paradigm with an inter-stimulus interval of 60 s was employed, using amyl-acetate as the stimulus. Subjects rated the perceived intensity of the stimulus on the Labeled Magnitude Scale, thereby facilitating cognitive processing of the olfactory information. EEG was recorded and averaged over 20 trials from three midline scalp electrodes (Fz, Cz, Pz) referenced monopolarly to linked earlobes. Between-session reliabilities for ERP component amplitudes and latencies were assessed as intra-class and Pearson correlation coefficients. Preliminary results indicate high reliabilities for N1, P2, and P3 latencies and N1-P2, N1-P3 interpeak amplitudes. The data suggests that the reliability of the OERP is comparable to that of auditory and visual ERPs, supporting the use of the OERP in domains of traditional ERP research. Supported by NIH grants DC02064 and AG04085 to CM.

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ODORANT DISCRIMINATION USING OLFACTORY EVENT-RELATED POTENTIALS

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The aim of these experiments was to determine whether the late positive component (LPC) of the olfactory event-related potential (OERP) could be used as an objective, nonverbal indicator of odorant discrimination. As example test materials, natural and synthetic menthol were used which were known to have different odors based on subjective verbal evaluations. To elicit the LPC, an oddball paradigm was employed where pairs of these odorants associated with different probabilities were presented to subjects (n =10). In each experiment, the high probability odorant (standard) was presented on 85% of the trials and the low probability odorant (target) was presented on 15% of the trials. In the first experiment, the standard was CO2 (50% v/v) and the target was natural menthol (10%). In the second experiment, the standard was synthetic menthol (10%) and the target was natural menthol (10%). In the third experiment, the standard was again synthetic menthol (10%) and the target was a mixture of synthetic menthol (5%) and natural menthol (5%). In each of the experiments, the subjects task was to press a button when the target was presented. Odorant discrimination decreased over the three experiments (i.e., more missed targets). Similarly, the size of the LPC decreased over the experiments.It is of interest to note that LPC's were even obtained to missed targets, suggesting that the LPC may be a more sensitive measure of odorant discrimination than subjective reports. It is concluded that OERP's are an objective, nonverbal and sensitive measure of odorant discrimination.

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MAGNETOENCEPHALOGRAPHIC ANALYSES OF CORTICAL GUSTATORY RESPONSES IN HUMANS

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Studies on non-invasive recordings from human brain have shown that the primary gustatory area (PGA) is located in the insular cortex and opecular area. The present study was designed to reveal characteristics of taste-elicited responses of the PGA by analyzing magnetic fields using a 122-channel whole-head neuromagnetometer. A computer-controlled stimulator delivery system was used for delivering taste solutions and rinsing water. Each taste solution was applied for 400 msec with the interval of 15 or 25 sec water rinse. In the first experiment, We used 50 mM citric acid, 500 mM sucrose, and 50 mM citric acid after chewing a piece of miracle fruit (Synsepalum dulcificum) that has a unique property of changing sour taste into sweet taste. The mean latency of responses to citric acid was shorter than that to sucrose, and that to citric acid after chewing a piece of miracle fruit was longer than that to citric acid and almost equivalent to that to sucrose. In the second experiment, subjects were stimulated with citric acid and sucrose in an alternate order or in a random order. Perceived intensity of the two stimuli was less salient and the evoked magnetic responses were smaller when applied randomly than alternately. The present study suggests that the perceived quality and intensity of taste stimuli are closely related with the activity of the PGA.

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ODOR-EVOKED CELLULAR ACTIVITY DETECTED BY FOS IMMUNOCYTOCHEMISTRY IS IN PRINCIPAL CELLS IN PIRIFORM CORTEX

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We have reported that odor-evoked cellular activity detected by immunocytochemistry for Fos protein has a broad, patchy distribution in anterior piriform cortex (APC) and a spatially dispersed distribution with no obvious patchiness in posterior piriform cortex (PPC; Illig & Haberly 2000). In view of the dominance of odor-induced c-*fos* activity in GABAergic cells in the olfactory bulb (OB; Guthrie et al. 1993), an important question is whether the patterns observed in piriform cortex represent activity in principal neurons or inhibitory neurons. To address this, we carried out dual immunocytochemical staining for Fos and GABAergic cell markers. Male hooded rats (~300g) were placed in an odor-free chamber for 15-18 h, then exposed to odor for 30 s intervals separated by 90 s (to minimize habituation) for one hour and rapidly perfused with fixative. Brain sections were stained for Fos and either calbindin, parvalbumin, cholecystokinin, or glutamic acid decarboxylase. Each of these substances colocalizes with GABA in one or more populations of inhibitory neurons in piriform cortex. Results show no double-labeling for Fos and any of the GABAergic markers, indicating that odor-evoked Fos-labeled cells in APC and PPC are excitatory cells. These findings suggest that odor-specific patches of Fos-labeled cells are groups of principal neurons and provide evidence for a transition from a precise modular representation of odor quality in the OB, through a loose patchy organization in APC, to a spatially distributed ensemble code in PPC. Supported by NIDCD grants DC00414 (KRI) and DC03271 (LBH).

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TRANSIENT EXCITABILITY CHANGES IN PIRIFORM CORTEX PRODUCED BY ACTIVATION OF CORTICAL ASSOCIATION FIBERS

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High-frequency stimulation (HFS) of association fibers (AF) in piriform cortex (PC) produces a rapid but transient potentiation of an early component in the field potential evoked by AF stimulation (Brain Res. 841, 27-42, 1999) and a brief change in the excitability of PC neurons (Soc. Neurosci. Abstr. 25, 2186, 1999). We investigated the intracellular mechanisms underlying these effects in male Long-Evans rats anesthetized with urethane (1.4 g/kg). Intracellular recordings were made from PC neurons using micropipettes containing 1 M KAc and 2% neurobiotin, followed by neurobiotin labeling. HFS of AF (10-pulse 100-Hz trains) induced two brief but dramatic changes in all PC neurons recorded (n=22): a hyperpolarizing shift in the membrane potential, and an increase in the amplitude of the intracellular depolarization evoked by AF stimulation. The duration of both effects was brief (typically < 1 min) and coincided with potentiation of the early component in the extracellular field potential. Analysis of reversal potentials indicated that the AF-evoked depolarization consisted of an EPSP followed by a depolarizing chloride IPSP, and that the amplitude increase was due primarily to an increase in the driving force on chloride due to membrane hyperpolarization. These results suggest that the predominant effect of AF stimulation in the PC is inhibitory, and that patterned activation of AF during sensory input may act to increase the signal-to-noise ratio in the PC by producing blanket inhibition of ongoing background activity. Supported by NIH grant DC02271.

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SIMULTANEOUS RECORDING OF EOG, OERP AND "BULBAR" POTENTIALS IN HUMANS

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The electro-olfactogram (EOG) is considered to be the summated generator potential of the olfactory receptor cells and therefore represents the peripheral olfactory events. On the other hand the olfactory event-related potential (OERP) represents both peripheral and central neuronal activity. The aim of the present investigation was to record the EOG and the OERP simultaneously in order to deconstruct the components of the OERP. To obtain additional information about the sequence of electrical events, a third recording site, on either side of the bridge of the nose, referred to as the "bulbar" site, was used. The electrical events recorded here should be intermediate between the EOG and the OERP. Electrical activity of the olfactory epithelium, the EOG, in response to odour pulses was recorded with a Ag/AgCl electrode (0.8mm dia) positioned in the olfactory cleft and attached to an input of an EEG machine. Pulses of amyl acetate (200ms, 10s intervals) were delivered by an olfactometer. No responses were obtained when control air was used or when the electrode was positioned in the nasal respiratory mucosa. OERPs (at Cz) and the "bulbar" potentials were recorded by standard EEG techniques. All the potentials were amplified and digitised and an average was created of sequential blocks of 10 trials. There was a temporal correlation between the EOG and OERP. The OERP waveform changed with time as did the rise-time of the EOG. The "bulbar" potential was similar in to the OERP with some subtle differences. Left and right "bulbar" potentials were similar in response to mono-rhinal stimulation.

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OLFACTORY TESTING WITH SNIFFIN' STICKS IN IDIOPATHIC PARKINSONISM

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Olfactory dysfunction seems to be one of the most frequent symptoms in idiopathic parkinsonian syndrome (IPS). In contrast to the increasing number of studies providing evidence of the usefulness of olfactory tests in the diagnosis of IPS, clinical assessment of the olfactory function is rarely performed. This may be due to the lack of an easy-to-use, inexpensive, standardized, and reliable test. In this study, we administered the newly developed Sniffin' Sticks test (or TDIT - threshold, discrimination, identification test) to a group of 40 nondemented IPS patients and compared the results with 40 healthy controls matched for age, gender, and smoking habits. In all three subtests including odor threshold (n-butanol), odor discrimination, and odor identification, the control group scored significantly better than the IPS group, yielding a specifity and sensitivity of 90% each. Our results also indicated that olfactory deficits in IPS can be found at early stages of the disease, appear bilaterally, and do not correlate with the dominant side of parkinsonism. Therefore, we emphasize that olfactory testing is an useful tool in the diagnosis of IPS.

SCREENING OF OLFACTORY FUNCTION USING A 4 MINUTE ODOR IDENTIFICATION TEST: RELIABILITY, NORMATIVE DATA, AND INVESTIGATIONS IN PATIENTS WITH OLFACTORY LOSS

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The study aimed to create a screening test on the basis of the odor identification test as used in the "Sniffin' Sticks" olfactory test kit. It should appeal to the practitioner in terms of (i) time required for testing, (ii) reliability, (iii) separation of "normal" from "abnormal", and (iv) it should allow lateralized screening. Experiments should provide a normative database (number of subjects >1000), establish test-retest reliability (n>100), and compare results from patients with olfactory loss (n>200). Correlation between results in two repetitive tests was .78. The test differentiated anosmics, hyposmics, and normosmics ($p<0.001$). None of 112 anosmics reached a score higher than 8; the 90th percentile was at a score of 6. When only 6 odors were used for calculating scores, for anosmics the 95th percentile was at a score of 4. These data provide the basis for the screening of patients using "Sniffin' Sticks".

DAILY LIFE IMPACT OF OLFACTORY LOSS

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Taste and smell are fundamental sensory systems essential in nutrition and food selection, for efficient metabolism, for the hedonic and sensory experience of the environment, and, in general, for the maintenance of a good quality of life. A sample of 278 consecutive patients with hyposmia or anosmia of any cause were examined by ENT-specialists, CT scan of the paranasal sinus and by a psychophysiological olfactory test. Additionally they were interviewed using a standardized self-reporting questionnaire. Dividing the sample into anosmic and hyposmic patients no major difference of subjective estimation of quality of life (QoL) could be detected. Depressive patients rated their QoL lower than patients without mood disorder, although their olfactory performance was about the same. Unchanged taste perception was only reported in 27% of hyposmic patients compared to 21% of anosmic patients. Respectively difficulties in cooking, eating of spoiled food, less appetite and too little perception of their body odor was reported by nearly half of our sample. Patients under the age of 55 years suffered more from the lower performance (reduction of QoL 40 vs. 33%). The most striking age specific problem seems to be body odor perception. With reference to the etiology of the chemosensory disorder at least one third of the patients (conducive disorders) could probably be helped and they could be relieved of their reduced quality of life and change in mood by surgical or medical treatment.

HEALTH SYMPTOMS FROM EXPOSURE TO ODOROUS CHEMICALS: PHYSIOLOGICAL VERSUS COGNITIVE EFFECTS

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Occupational exposure to some odorous volatiles can elicit sensory and physiological responses leading to health symptoms in workers. In contrast, some health symptom reports may be cognitively mediated through the perception of odor. An exposure study was conducted to determine the relative impacts of physiological versus cognitive effects on health symptom reports. Twelve workers, with regular occupational exposure to Isopropyl Alcohol (IPA), were compared to 12 controls during three 4-hour exposure conditions: IPA (400 ppm), Odor-only (PEA), and No-odor (clean air). Objective endpoints of ocular and nasal irritancy (e.g., nasal patency, nasal secretion and ocular hyperemia) and subjective health symptom ratings were obtained at baseline and following two and four hours of exposure. Ratings of odor, irritation and annoyance intensity were obtained throughout the exposure. Odor intensity was rated most intense in the IPA condition, followed by the Odor-only and the No-Odor condition; however, rated irritation and annoyance were significantly higher only during exposure to IPA. Neither the objective endpoints nor subjective health symptoms differed between exposure conditions and groups. Overall, we found that rated irritation during short-term exposure to IPA was significantly correlated with ratings of annoyance and odor intensity, suggesting that reported irritation from IPA may be mediated by hedonic reactions to its olfactory properties.

OLFACTORY DEFICITS IN ALCOHOLICS

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Despite the interest in the olfactory deficiencies associated with Korsakoff's syndrome, there has been little systematic assessment of olfactory functions in nonamnesic and nondemented alcoholics. However, few findings indicate that alcoholism is associated with olfactory identification impairments which have been shown to be only partially reversible with abstinence and that cortical structures play an important role in this sensory loss. The aim of the present study was to assess various olfactory functions in alcohol dependent patients. Olfactory evaluation was performed with 30 alcohol dependent patients (DSM-IV) and 30 healthy controls, matched for age, sex and smoking status. Following a multivariate approach of olfactory functioning we used the "Sniffin' Sticks" for screening multiple types of olfactory measures. This test comprises three tests of olfactory function, namely test for odor threshold, odor quality discrimination and odor identification. The results demonstrate that alcoholics are impaired on olfactory acuity (odor threshold), odor quality discrimination and odor identification. Patients and controls did not differ in general intellectual ability as assessed by basic neuropsychological tests: the Mehrfachwahl Wortschatz Test (MWT-B) and the Mini-Mental State Examination (MMSE). Duration of dependency and length of abstinence prior to testing had no essential effects on olfactory functions. Our results provide evidence that chronic alcoholism has detrimental effects on olfactory performance, even on olfactory functions which presumably require less cognitive processing than does odor identification.

PRIMING EFFECT ON OLFACTORY FUNCTIONS IN PATIENTS WITH SEASONAL ALLERGIES

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It has been reported that patients with seasonal nasal allergies exhibit olfactory loss during the peak season but also when measured out of season. It has been described that one nasal allergy causes more severe symptoms which is known as the priming effect. The aim of our study was to revalidate the olfactory sensitivity of allergic patients in view of different olfactory functions measured out of peak season and to investigate the consequences of the priming affect to the olfactory acuity. We examined 139 patients who were all desensitised divided into three groups: Group 1 – 63 patients to birch or grass pollen, group 2 – 43 patients with birch and grass pollen allergy, and group 3 - 29 patients with birch and grass pollen and a third allergy. Olfactory testing was performed using the validated kit "Sniffing Sticks" which includes assessment of odour threshold (T), odour discrimination (D) and odour identification (I) compared with age and sex matched normative data of healthy subjects. The mean olfactory sensitivity of patients with one or two seasonal allergies outside the peak season was not different from healthy subjects maybe as a consequence to the hypo sensitisation. Patients with three nasal allergies showed a significant loss of olfactory acuity. We found significant lower values of odour thresholds in patients with more than two seasonal allergies while odour discrimination and identification did not vary. This might indicate a cumulative impairment.

A NEW SCREENING TEST FOR OLFACTORY DISORDERS BASED ON RETROPHARYNGEAL SMELLING

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We developed a fast and easy to use screening test for smell disorders using the principal of retropharyngeal smelling of aromas. The test is based on the use of aromatized sorbit candies (500 mg). Subjects were instructed to identify 23 different aromas using 4 items in a multiple choice test. In a first part we investigated 50 female and 50 male subjects (age 21 to 85 years) in three separated sessions on different days. The olfactory performance of each subject was tested using the validated Sniffin`Sticks on one of the three days. In the other two sessions the new test system was used to define the test-retest reliability and to compare results with the Sniffin` Sticks scores. In a second part patients with different olfactory disorders were additionally investigated using data from olfactory evoked potentials in order to differentiate their deficits. The test-retest reliability was rather high (r = 0.83). There was no sealing effect. Also, the correlation between Sniffin`Sticks and the new test was sufficiently good (r = 0.805). In combination with OEP data we could demonstrate that the new test helps to discriminate hyposmic, ansomic and parosmic patients. In the future we want to explore the usefulness of the test in pediatrics and for epidemiological investigations.

IMMUNOCYTOCHEMICAL AND FUNCTIONAL IDENTIFICATION OF PACAP IN DEVELOPING AND ADULT OLFACTORY EPITHELIUM

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Pituitary adenlyate cyclase activating peptide (PACAP) is found throughout the CNS and in peripheral organs, and is an important regulatory peptide, especially in sensory systems.[1] In development, PACAP's effects are largely neurotrophic while in adult neurons, PACAP is neuroprotective.[1] PACAP is present at relatively high levels in both developing and adult olfactory bulb. Surprisingly, PACAP has not been looked at in the peripheral olfactory system. Our immunocytochemical studies show that PACAP is differentially distributed in developing compared to adult rodent olfactory mucosa. Confocal imaging of fluo-4 loaded slices from olfactory epithelium of neonatal mice shows that ORNs respond to 100 nM PACAP-38 with increases in intracellular calcium (3%-35% increase in $\Delta F/F$; n=12). Fura-2 loaded ORNs from adults also show PACAP-induced calcium rises (130% ± 40% increase in fluorescence ratio; n=14). In some cells, the calcium increase is via release from caffeine-insensitive intracellular stores (114% ± 19%; n=5). While multiple responses can be observed in ORNs, the responses desensitize in the maintained presence (120 s) of PACAP. Our data represents the first identification of PACAP in peripheral olfactory epithelium and demonstrates the presence of functional PACAP receptors on ORNs. We postulate that PACAP plays a neurotrophic role in neonatal ORNs and during turnover in adults, and a neuroprotective role in adult ORNs. Supported by NIH NIDCD DC02994. 1. A. Arimura, Jpn.J Physiol 48:301 (1998).

ONTOGENY IN OLFACTION: COMPARISON OF TURNOVER IN THE OLFACTORY SYSTEM OF POST-LARVAL AND ADULT SPINY LOBSTERS

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Continuous turnover of neurons occurs in the olfactory organ and brain of many animals, including crustaceans such as spiny lobsters *(Panulirus argus).* This turnover includes proliferation and death of olfactory receptor neurons (ORNs) in the antennule and local and projection olfactory interneurons (OIs) in the brain. We have examined the ontogeny of turnover by studying 2 stages in the life of spiny lobsters: post-larvae (=post-pueruli) and young adults. Turnover was examined by quantifying changes in the number of i) olfactory sensilla added after molting and ii) ORNs and OIs added, by BrdU labeling. Turnover is similar in the 2 stages in that new ORNs and OIs are continuously added in specific proliferation zones. However, post-pueruli have a greater proportional of proliferating cells and less cell death than do adults. Although both post-pueruli and adults have ~8% increase in body size after molting, the net increase in ORNs is ~40% in post-pueruli but only ~4% in adults. In post-pueruli, this increase solely reflects ORN proliferation, since cell death is rare at this stage; in adults, the net increase reflects both proliferation of new ORNs (~18% increase) accompanied by death of old ORNs (~14% loss). Proliferation rate of OIs in the brain is also relatively much higher in post-pueruli than adults. Thus, turnover in the peripheral and central olfactory systems occurs throughout the life of spiny lobsters, but the rate of proliferation changes as does the balance between proliferation and cell death. Supported by NIH DC00312 & NSF IBN 0077474.

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MOLECULAR CLONING, CHARACTERIZATION, CELLULAR LOCALIZATION AND POSSIBLE FUNCTION OF A NOVEL CUB SERINE PROTEASE IN THE OLFACTORY ORGAN OF THE SPINY LOBSTER PANULIRUS ARGUS

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A novel gene encoding a protein with high homology to trypsin-like serine protease and CUB was identified from a spiny lobster olfactory cDNA library. The full length cDNA is 1802bp, encoding a protein of 50.25kD, with 3 domains: signal peptide, trypsin-like serine protease, and CUB. RT-PCR, Northern blot and Western blot showed that this protein is predominantly expressed in antennular lateral flagellum and eye, but not in other organs (brain, muscle, antennular medial flagellum, hepatopancreas, intestine, leg); trace expression may be present in second antenna and leg tips. Immunocytochemistry showed that in the lateral flagellum, this CUB-serine protease is highly expressed in olfactory aesthetasc sensilla: around glia (auxiliary cells) surrounding the inner dendrites of olfactory receptor neurons (ORNs), and around the ORN outer dendrites in the receptor lymph. We propose that this protein is expressed and secreted by glial cells, associates with ORN cell membranes or extracellular matrix via the CUB domain, and has trypsin-like activity. It may function in: i) perireception (activation or inactivation of odorants), ii) regulation of growth or function of ORN dendrites, or iii) facilitating molting. In the eye, this protein is not associated with sensory neurons or cuticle, suggesting a different function. To our knowledge, this is the first report of a gene encoding a protein with serine protease and CUB domains in any olfactory system. Supported by NIH DC00312 and NSF IBN 0077474.

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A STUDY OF MAMFAS II EXPRESSION IN THE NORMAL AND LESIONED RAT OLFACTORY SYSTEM

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mamFas II, also known as OCAM, RNCAM (for RB-8), and N-CAM 2, is expressed in the mammalian nervous system as both TM and GPI-linked forms. In olfactory epithelium (OE), it is expressed at high levels by neurons of Zones II and III and at low, but detectable, levels in Zone I neurons. We have studied the expression of the two forms of rat mamFas II in normal, newborn, bulb-ablated, and MeBr-lesioned rats by sequence analysis, Northern blot, RPA, and ISH. The results indicate that mamFas II is more closely related to Drosophila Fasciclin II than NCAM is, in terms of isoform similarity and sequence homology. mamFas II is widely, but differentially, expressed in the nervous system including cerebral cortex, thalamus, brainstem, cerebellum, spinal cord, OE and OB, etc. However, there are differences in the pattern of expression as a function of neuronal maturity. The short TM transcript (3.6kb) is the principal form in normal OE, elsewhere the long TM transcript (7.8kb, differing in the 3' UTR) predominates. OE composed of immature neurons has even higher short/long TM ratio. Furthermore, immature olfactory neurons also tend to express the GPI form at a higher relative level. Finally, we find that mamFas II is expressed by olfactory glia and that level are higher in ventral and lateral ONL than in dorsal and medial, which corresponds to the part of the ONL traversed by strongly mamFas II-(+) fibers. Thus, we conclude that the expression of the isoforms of mamFas II is regulated by the maturational state of the neuron. Supported by DC00467.

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OLFACTORY PLACODE DEVELOPMENT AND CELL DEATH IN THE MASTERBLIND MUTANT

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In order to understand the cellular events giving rise to the olfactory placode we have analyzed the masterblind (mbl) mutant (Heisenberg et. al., 1996). This mutant is characterized by the lack of olfactory placodes and optic vesicles, reduced telencephalon, and an expanded epiphysis. We examined the expression pattern of the distal-less-3 (dlx-3) gene, which is expressed in the developing nose and ear. In the mbl mutant dlx-3 expression was normal in the ear, whereas expression was reduced in the olfactory placode field. To determine whether the loss of dlx-3 expression was due to cell loss we assayed cell death using fluorescein TUNEL labeling. While cell death was not concentrated in the region of dlx-3 expression, it was increased in the mutant. The increased cell death in mbl, as compared to their wild-type siblings, was concentrated in the forebrain, epiphysis and jaw region. Previously we made the observation that the mbl phenotype varies with genetic background, where the severity is decreased on the "AB" wild-type background (mbl /AB) as opposed to on the "TL" background (mbl /TL). In order to separate the mbl phenotype from the background effect of cell death, we blocked cell death using Z-FAD-fmk, a known caspase inhibitor. Preliminary results showed a partial rescue of the morphological phenotype in the "TL", or more severe, background. Therefore, the mbl /TL phenotype is due in part to cell death caused by genetic background. We are now determining whether blocking cell death in mbl can rescue dlx-3 expression in the early embryo.

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EXPRESSION OF GALECTIN-1 AND GALECTIN-3 IN THE HUMAN VOMERONASAL ORGAN

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We investigated the distribution of galectin-1 (Gal-1) and galectin-3 (Gal-3) in the human vomeronasal organ using immunohistochemistry. For comparison, we also stained the olfactory epithelium of human specimens and postmortem biopsies. Gal-1 was expressed in the subepithelial connective tissue and in basally located cells of the vomeronasal epithelium (VNE). Gal-3 occurred in the cytoplasm of selective bipolar cells of the VNE and mucous glands. In the olfactory epithelium, Gal-3 co-localized with mature olfactory neurons identified by activity for Olfactory Marker Protein (OMP), which were not detectable in the VNE. The effects of both endogenous lectins range from apoptosis to cell maturation and might appear to act in an antagonistic manner, dependent on the physiological activity of the tissue entity. Both lectins have been hypothesized to be involved in cell differentiation processes. The similar expression and distribution of Gal-1 in the VNE and olfactory epithelium indicates a similar differentiating activity of both basal cell populations, but a different, non-neuronal development of the VNE as demonstrated by the different Gal-3 expression.

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NOGGIN AND BMP EXPRESSION IN THE SEL OF ADULT MICE

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Neurogenesis in the olfactory bulb of rodents persists far beyond the postnatal period. Recently, this neurogenesis has been related to the migration of cells generated in the subependymal layer of the lateral ventricle (SEL). The newly formed cells migrate tangentially along the SEL rostral extension, as chains of highly undifferentiated elements within the glial tubes. These tubes represent an arrangement of the astrocytic glia separating the migrating neuroblasts from the mature brain tissue. Whereas a number of studies have analyzed the SEL from a morphological and molecular point of view, the mechanisms regulating neurogenesis in this area are not fully understood. Here, by using a noggin-LacZ heterozygous mouse, we have investigated the expression of noggin and different bone morphogenetic proteins (BMPs) in the SEL of adult mice. These secreted factors are implicated in early neural development. Our results demonstrate the occurence of noggin, BMP4 and BMP7 all along the SEL, from the lateral ventricle to the olfactory bulb. Double immunostaining for these molecules and markers of glial tubes (GFAP) and migrating neuroblasts (PSA-NCAM) show that noggin, BMP4 and BMP7 are primarily associated with the glial compartment. With regard to the role played by these factors during neural development, and the expression patterns shown here, we hypothesize that the astrocytic cells of the glial tubes contribute to mantain an embryonic-like environment allowing neurogenesis all along the SEL area. NIH DC00347(FLM).

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NADPH DIAPHORASE IS DEVELOPMENTALLY REGULATED IN RAT OLFACTORY EPITHELIUM

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In vertebrate olfactory receptor neurons, NO synthase (NOS) has been detected in embryonic and early postnatal stages, but its expression in the mature epithelium is controversial. We analyzed the distribution of the NOS-marker NADPH diaphorase (NADPHd) in the olfactory epithelium of rats at nine developmental stages. NADPHd was expressed in a small subset of olfactory neurons as early as P0. After P0, the number of NADPHd-positive neurons increased rapidly, reaching a maximum about one month later. The density of labeled neurons rose tenfold during that period. Whereas the NADPHd reaction predominated in the somata and in the nuclear region, a strong transitory staining could be observed in the dendrites and olfactory knobs between P2 and P5, diminishing thereafter. To characterize the population of NADPHd-positive neurons, we prepared double labelings of NADPHd with anti-olfactory marker protein (OMP) immunochemistry, which labels mature olfactory neurons. Both NADPHd and OMP stained cells from the middle zone of the epithelium, where the somata of the mature neurons reside, but there was no evidence for a linked expression of the two markers. These data display the dynamic postnatal regulation of the cellular distribution of NADPHd, which appears to reflect developmental processes within the olfactory epithelium. Supported by Fondecyt 1990938, Presidential Chair in Science and ICM P99-031-F (JB).

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A SINGLE FUNCTIONAL UNIT IN RAT OLFACTORY BULB: FUNCTIONAL MRI STUDY

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Results from various methods support the concept that odor information is presented in the olfactory bulb (OB) by activity patterns of individual functional units, glomeruli. We have recently applied functional MRI (fMRI) to map the odor-elicited patterns in the whole rat OB (Xu et al, PNAS, 2000). Here we present functional MRI data of the rat OB with improved spatial resolution of 110x110x125 μm^3, which is at the level of a single glomerulus. To locate activities in the bulbar laminar structures, anatomical images were obtained with resolution of 55x55x125 μm^3. The laminar structures revealed by MRI and histological staining correlate well in the same rat, providing the anatomical basis for the interpretation of the fMRI data. Functional MRI images were obtained with ~2 minutes of isoamyl acetate exposure. The activity patterns at high resolution correlate very well with lower resolution (i.e. 220 x 220 x 250 μm^3) at the same position in the same animal. Similar to the lower resolution results, the highly activated pixels frequently form clusters across the laminar structures, although random 'hot-spots' do exist, and the patterns are highly reproducible over several hours. However, with higher spatial resolution, the activation of single glomerulous can be detected and the activity in the olfactory nerve layer can be distinguished clearly from the activity in the adjacent glomerular layer. Supported by NIH grants DC-003710 (RGS), NS-37203 (FH) and an NSF grant DBI-9730892 (FH). .

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MOLECULAR DISSECTION OF URINE ODOR EVOKED RESPONSE IN THE MAIN OLFACTORY BULB

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Odor-induced neuronal activity patterns in the OB are believed to originate in the differential odor responses of olfactory receptor neurons, which synapse on mitral and tufted cell dendrites in olfactory bulb glomeruli. Olfactory receptor mRNA has been shown to be present in olfactory receptor neuron axons making it theoretically possible to clone olfactory receptors involved in a specific odor response. In order to understand the mechanisms responsible for in vivo discrimination of a biologically meaningful odor stimulus we have generated an olfactory receptor library from glomeruli responsive to urine. Awake female mice were exposed to novel male urine odor. The neural activity patterns evoked within their main olfactory bulbs were mapped by measuring increases in c-fos mRNA, a marker for neuronal activity, in periglomerular cells. Coordinates for tissue microdissection and subsequent cloning were determined by defining areas with the highest urine-evoked activity. Thirteen different receptors with sequence similarity to the olfactory receptor family were identified. Antisense cRNA probes made from two of these receptor cDNAs hybridized to the areas we microdissected. These results suggest that it is possible to generate olfactory receptor libraries with known odor specificities by a combination of c-fos mRNA activity mapping and RT-PCR.

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INHIBITION OF BURST FIRING AND BACKPROPAGATING ACTION POTENTIALS IN THE DENDRITES OF MITRAL CELLS

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In the mammalian olfactory bulb, GABAergic inhibition of mitral cells, mediated by reciprocal dendrodendritic synapses with granule cells, transforms odor-specific patterns of glomerular activity into spatiotemporally patterned mitral cell activity. This study examined the spatial range of inhibition, and its effect on backpropagating bursts of action potentials in mitral cell dendrites in bulb slices from P21-30 rats. Using laser photostimulation, functional GABA receptors were mapped on the mitral cell soma, axon and dendrites. Inhibitory range in the secondary dendrite, estimated by termination of firing by local photolysis, varied with the ratio of excitation to inhibition. Under synaptic block, dual patch recordings revealed spike bursts (~50Hz) actively backpropagating into the secondary dendrites with uniform activity-independent attenuation, subject to amplitude modulation and reflection by focal GABA photostimulation. A dynamic radial domain model is proposed for inhibitory input processing in the secondary dendrite: input to a proximal domain controls temporal coding, while input to an extensive distal domain shapes spatial coding by modulating voltage waveforms, potentially altering long range patterns of Ca^{2+} influx and dendrodendritic transmission. Local dendritic processing can switch dynamically between temporal and spatial modes. These mechanisms may produce polarized patterns of lateral inhibition, and contribute to the transformation of spatial codes and synthesis of temporal codes in the bulb. Supported by a gift from the Monell Foundation, & NIDCD DC00505-12.

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GLUTAMATE TRANSPORTERS AND ODOR PROCESSING IN THE OLFACTORY GLOMERULUS

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Glutamate transporters play a vital role regulating extracellular concentrations of glutamate in the CNS and PNS. These membrane-bound, sodium-dependent glycoproteins are expressed in astrocytes or neurons where they perform high-affinity binding and uptake of glutamate from extracellular spaces. Glutamate uptake, or lack of it, affects interneuronal communication and neuronal health. Interneuronal communication in odor processing first occurs in the glomeruli of the olfactory bulb (OB) and involves a glutamatergic synapse between olfactory nerve terminals and OB neurons. Using immunohistochemistry and confocal, light and electron microscopy, I have determined the patterns of glutamate transporter expression in the mouse OB. The glial transporter GLAST was abundant in all layers of the bulb, especially the glomerular layer where GLAST positive glial processes circumscribed large (6-14 m) GLAST negative, irregularly shaped spaces. These GLAST negative spaces consisted of synaptic nests formed by olfactory nerve terminals and dendrites, as shown by TEM. The other glial transporter, GLT-1, and the neuronal transporter EAAC1 were minimally present. GLT-1 was most abundant in the internal plexiform layer; EAAC1 was expressed in the cell bodies of granule cells, mitral and tufted cells, but not to any great extent in their dendrites. These results indicate that, while GLAST is responsible for the majority of glutamate uptake in the OB, the lack of GLAST in glomerular synaptic nests may promote glutamate diffusion there. Glutamate diffusion could play an important role in odor processing in the olfactory bulb.

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NITRIC OXIDE MODULATES ODOR-EVOKED ACTIVITY PATTERNS IN THE GLOMERULI OF THE MOTH ANTENNAL LOBE

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There is growing evidence that nitric oxide (NO) functions as a gaseous messenger in the nervous systems of both vertebrates and invertebrates. In the moth *Manduca sexta*, anatomical characterization of the NO/sGC signaling system strongly suggests that NO released from sensory afferents modulates the activity of odor-information processing circuits in the antennal lobe. In order to demonstrate this modulation, we have examined the effects of changing NO levels on the response properties of different classes of olfactory interneurons that innervate the glomerular neuropil. Our results showed that: 1) reducing normal NO levels (by treating with carboxy-PTIO or L-NAME) led to clear and consistent changes in the odor-evoked activity patterns of both local interneurons (LNs) and projection neurons (PNs), and 2) NO depletion can increase or decrease neural excitability depending on which neurons in a particular circuit are being monitored. One mechanism underlying these effects may be related to the regulation of chloride conductances in some PNs. In other neurons, NO seems to play a role in modulating resting potential. Either of these mechanisms would result in a substantial change in the odor-evoked activity patterns in glomerular output pathways. These results therefore suggest that the pattern of activation evoked by a given odor is not fixed, but subject to modulation according to changes in endogenous levels of NO.

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SYNCHRONOUS BURSTING AMONG JUXTAGLOMERULAR NEURONS OF THE RAT MAIN OLFACTORY BULB (MOB) IN VITRO

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Olfactory nerve (ON) terminals interact synaptically with local circuit and projection neurons within the glomerular layer (GL) of the MOB. The GL contains distinct populations of juxtaglomerular (JG) cells, including periglomerular (PG), short axon (SA) and tufted (T) neurons. Using single and paired recordings in MOB slices, we investigated spontaneous and ON-evoked activity of JG neurons that were filled with biocytin and reconstructed. T cells in the GL (n=14) exhibited bursting discharge of 2-5 bursts/s, each burst consisting of 2-8 spikes. These neurons had dendrites that ramify largely throughout a single glomerulus. They all responded to ON stimulation with a single constant latency excitatory postsynaptic current (EPSC). Highly synchronous bursting discharge was recorded from pairs of T cells whose dendrites extend into the same glomerulus. PG (n=11) and SA (n=7) cells did not spike at resting membrane potential, but exhibited spontaneous bursts of EPSCs at 2-5 Hz; ON stimulation also evoked bursts of EPSCs in these cells. Recording from close pairs (n=5) of T and PG/SA cells showed that bursts of T cells spikes were 60-80% synchronous with PG/SA cell EPSC bursts. Therefore, spontaneous activity is highly correlated among T cells whose apical dendrites ramify within the same glomerulus, and among adjacent pairs of T and PG/SA cells. This synchronous activity occurs at theta frequency characteristic of investigative sniffing in rodents, and may play an important role in olfactory coding. PHS grants: DC03195,DC02588,DC00347 & NS36940.

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CONTRIBUTION OF CYCLIC-NUCLEOTIDE-GATED CHANNELS TO THE RESTING CONDUCTANCE OF OLFACTORY RECEPTOR NEURONS

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During transduction in olfactory receptor neurons (ORNs), cytoplasmic cAMP gates cyclic-nucleotide-gated (CNG) channels, resulting in neuronal depolarization. Whether cAMP also gates CNG channels in unstimulated ORNs is unknown. We studied the effects of divalent cations, adenylate cyclase inhibitors, and cAMP analogues on the resting slope conductance of isolated ORNs of the grass frog (*Rana pipiens*). Slope conductances were measured between -85 mV and -55 mV under whole-cell and perforated-patch recording. Extracellular divalent cations (2 mM Ca^{2+} plus 1 mM Mg^{2+}), which block the CNG channels, significantly and reversibly reduced the slope conductance by 40% (n=12). The membrane-permeant adenylate cyclase inhibitor ML12330 (1 or 10 μM), significantly decreased the conductance by 20% (n=13). Another such inhibitor, THFA (100 μM), also significantly reduced the resting conductance of the cells (20% reduction, n=6). The effects of cAMP analogues introduced directly into the cell via the recording pipettes were mixed. ATP (200 μM, n=8) and ATP-γ-S (100 or 200 μM, n=7) had no significant effects. One ATP analogue, AMP-PNP (200 μM), did cause a significant decrease in conductance (n=7). Our results suggest that unstimulated ORNs contain sufficient cAMP to activate the CNG channels. The resting CNG conductance accounts for 20-40% of the input conductance measured in an unstimulated ORN. Having a low level of cAMP at rest may leave the neuron poised to give a bigger response to a small stimulus-activated increase in cAMP level. This work was supported by NIH grant R01 DC00926.

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CHEMOSIGNAL-ACTIVATED WHOLE-CELL CURRENTS IN THE VOMERONASAL (VN) ORGAN

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A total of 198 patch-clamp, whole-cell recordings were made from 42 *Sternotherus odoratus* (stinkpot/musk turtle) to study the electrical- and chemosignal-activated properties of vomeronasal (VN) neurons. Introduction of dextran tetramethylrhodamine into the VN orifice permitted good initial characterization of the organ and central projections prior to its dissociation to single neurons. Basic electrical properties were measured (resting potential = 54.5 ± 2.7 mV, input resistance = 6.7 ± 1.4 GΩ, capacitance = 4.2 ± 0.3 pF). Neurons were held at Vm = -60 mV, stimulated for 700 ms with 5 natural stimuli, and responded to at least 1 of the 5 chemosignals in 33 of the 90 cells tested (34%). The peak amplitude chemosignal-evoked currents ranged from 4 to 180 pA, with 2/3 of responses ≤ 25 pA. Currents were of either polarity, depending upon the cell and the chemosignal tested. Musk-evoked inward currents were 3 fold the magnitude of urine- or catfish-extract-evoked inward currents. Urine applied on neurons harvested from female animals evoked currents that were 2-3 fold larger than those elicited from male neurons. The calculated breadth of responsiveness (H metric = 0.088) for neurons presented with this array of 5 stimuli indicated that the response spectra of the VN neurons is narrowly tuned. Second-messenger signaling is being explored given our identification of IP_3-R type III in a protein-protein interaction complex with trp2. Supported by MBL Summer Research Fellowships, DC00378, & T32-DC00044.

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IONIC CURRENTS AND ODOR RESPONSES OF CULTURED OLFACTORY RECEPTOR NEURONS FROM ANTENNAE OF THE HONEYBEE

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It has proven possible to establish dissociated cultures from prepupal antennal cells of the honeybee (Gascuel et al,1994) demonstrating morphological and ultrastructural differences between neurons and other cells. We recorded electrophysiological properties and odor-evoked reponses from presumptive olfactory receptor neurons (ORNs) from drone and worker prepupal antennae. In voltage-clamp depolarizing steps elicited at least a transient inward current followed by outward currents with transient and sustained components. The inward current was a sodium current and sensitive to tetrodotoxin. The outward currents consisted of a voltage-activated, sustained K current with characteristics similar to those of the delayed rectifier and a transient outward current which was Ca-dependent. Upon depolarizing current steps action potentials (AP) were recorded from the cell bodies as early as 2 days in culture revealing two different types of response pattern, some neurons generated multiple APs with increasing current densities, while others generated only one AP. Application of a given odor stimulus, single odors and their mixtures, induced prolonged transients membrane depolarizations on which very often were superimposed a train of APs. More than 90% of the ORNs of the worker bee responded to at least one mixture or a single odor. The ORNs from drone reponded to 83% to a blend of queen pheromone In summary, ORNs of worker and drone bees posses voltage- and odor-activated currents very early in their embryonic development. .

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PHOSPHATIDYLINOSITOL 3-KINASE-DEPENDENT SIGNALING IN RAT OLFACTORY RECEPTOR NEURONS

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Acutely dissociated Wistar rat olfactory receptor neurons loaded with the calcium indicator Fura 2-AM responded to a mixture of 100 odorants with a transient increase in [Ca2+]i. The odorant-evoked increase in [Ca2+]i was greater following pretreatment with 50 μM LY294002, an inhibitor of phosphatidylinositol 3-kinase (PI3K). Included were cells that only responded to the odorant following pretreatment with the drug. No cells responded to the drug in the absence of the odorant. Phosphatidylinositol 3,4,5-trisphosphate (PIP3)-AM rescued the effect of LY294002. Another inhibitor of PI3K, 50 μM Wortmannin, mimicked the effect of LY294002. Pretreatment with up to 100 μM U73122, a specific inhibitor of PLCβ, enhanced the effect of blocking PI3K. Pretreatment with 50 μM MDL12330A, a specific antagonist of adenylyl cyclase, blocked the response to the odorant. We interpret these findings to indicate that activation of the 3-phosphoinositide (PI) pathway, probably in concert with the canonical PI turnover pathway, inhibits cyclic nucleotide-dependent excitation, implying that PI signaling acts together with cyclic nucleotide signaling to encode odor information in rat ORNs.

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CO_2-SENSITIVE OLFACTORY RECEPTORS IN RATS

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Amphibians, reptiles, and mammals have been shown to possess CO_2-sensitive olfactory receptors that mediate a decrease in ventilation when stimulated by low concentrations of CO_2. The objective of the present study was to locate sites in the rat olfactory epithelium exhibiting an electrophysiological response to CO_2 and to determine the dose-response characteristics of these receptors. Seven rats of both sexes, ranging in age from P15-P300, were prepared for surgery and for recording electro-olfactograms (EOG) from the olfactory epithelium, according to previously described methods (Scott et al., 1997). We tested 141 sites on the olfactory epithelium and found that 7 sites, located in the medial and caudal regions of endoturbinates II and II', exhibited an EOG response to CO_2. In most cases, CO_2 concentrations as low as 2% evoked an olfactory receptor response. The average EOG response amplitude exhibited a dose-dependent increase for CO_2 above 2%, reached a maximum around 14-16% CO_2, and showed a slight decrease in response above 16% CO_2. These data show that like CO_2-sensitive olfactory receptors in amphibians, rat olfactory CO_2 receptors are stimulated by physiological concentrations of airway CO_2. In addition, because the rat olfactory CO_2 receptors reached a response maximum around 14-16% CO_2 it appears that olfactory CO_2 receptors may be selectively stimulated by low concentrations of CO_2 whereas noxious levels of nasal CO_2 (45-100%) stimulate trigeminal nerve endings located in the nasal epithelium (Thürauf et al., 1991; Kobal and Hummel, 1994). Supported by NIH grant HD 37381-01 from the NICHD.

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CL AND K CURRENTS UNDERLIE HYPERPOLARIZING RECEPTOR POTENTIALS IN LOBSTER OLFACTORY RECEPTOR NEURONS

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To further understand the ionic basis of odorant-evoked inhibition in lobster olfactory receptor neurons (ORNs), we investigated whether activation of a 4-aminopyridine-blockable K current (Michel, et al., *J. Neurosci.*, 65:446-53,1991) could account for inhibitory receptor potentials in all cells. We present data suggesting that odorants can also inhibit lobster ORNs by suppressing a steady-state Cl conductance. The hyperpolarizing receptor potential in a population of current-clamped lobster ORNs *in situ* (Em=–70 mV) was associated with a decrease in membrane conductance of 24±2% (±SEM, n=11), was reduced or eliminated by 9-AC (n=10), reversed polarity at –22.3±3.7 mV (±SEM, n=32), close to the calculated E_{Cl^-}, and was insensitive to lowering $[Cl^-]_o$ during the odorant response (n=5). Preliminary pharmacological evidence indicates that this 9-AC blockable Cl- conductance can coexist with the 4-aminopyridine-blockable K+ conductance reported earlier, suggesting that two distinct cellular mechanisms can mediate odorant-evoked inhibition in lobster ORNs. Supported by the NIDCD (DC01655).

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BIOSYNTHESIS AND LOCALIZATION OF TAURINE IN THE NASAL MUCOSA

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The amino acid taurine is a potent neurotrophic and cell-protecting factor that is required for maturation and differentiation of developing nervous tissue. Olfactory sensory neurons, because of their proximity to the external environment and the fact that they are continually replaced throughout life, may be particularly influenced by the presence of high taurine concentrations found in the nasal mucosa. Knowledge of the nature of mucosal cells producing and accumulating taurine is essential for investigating its physiological role in olfaction. This study sought to localize taurine and cysteine sulfinate decarboxylase (CSD; EC 4.1.1.29), the rate-limiting enzyme of taurine biosynthesis, in the rat nasal mucosa. Expression of the enzyme was examined using antiserum against a synthetic peptide that was designed from the CSD sequence (residues 44-57). Western blot analysis of proteins extracted from the nasal mucosa demonstrated that, following affinity purification of the IgG fraction, antibodies recognize a protein with a molecular mass similar to that of the CSD protein (about 55 kDa). Immunoprocessing of paraffin sections indicated that staining for CSD and taurine is localized in cell bodies, dendrites, and axons of olfactory sensory neurons. The results suggest that only sensory cells produce and accumulate taurine in nasal mucosal tissue. Supported by NIH grant DC04083 (I.K.).

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PRIMARY CULTURE OF LOBSTER (*HOMARUS AMERICANUS*) OLFACTORY RECEPTOR NEURONS

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In order to develop a model system where the necessity of specific proteins in lobster olfactory transduction can be tested, we have investigated conditions necessary for primary culture of lobster olfactory receptor neurons (ORNs). Spiny lobster (*Panulirus argus*) ORNs survived longest when plated in L-15 media on poly-D-lysine coated coverslips or on hemolymph clots (Fadool et al., 1991. Tissue and Cell 23:719). We cultured *H. americanus* ORNs in L-15 media and varied 1) temperature, 2) fetal bovine serum, hemolymph, and basic minimal essential vitamin supplementation, and 3) substrate (Falcon Primaria plastic, poly-D-lysine, or hemolymph clots). The ORNs did best on poly-D-lysine at 5°C supplemented with vitamins and hemolymph, with cells surviving at least 12 days. The whole-cell patch clamp technique was used to test whether these cultured cells exhibited properties of lobster ORNs. Depolarizing voltage steps from a holding potential of -60mV elicited a transient inward current followed by a much larger outward current. Five out of eleven cells tested with a complex odor mixture prepared from TetraMarin extract responded with an inward current. These properties are characteristic of lobster ORNs and were present out to at least 6 days in culture. We are now testing methods for transfecting the cultured ORNs with cDNA or protein.

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PATCH-CLAMP RECORDINGS FROM HUMAN OLFACTORY NEURONS

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Considerable progress has been made in characterizing the basic response properties of olfactory receptor neurons (ORNs) using many animal models. While G-protein coupled receptors linked to second messenger pathways appears to be a common theme for signal transduction, differences in the specificity and initial processing of signals in ORNs clearly exist among species. To identify these processes in humans, we have recorded from 81 freshly isolated human ORNs isolated from biopsies using the Gramicidin perforated-patch technique. Families of whole cell currents generated under voltage clamp were used to characterize voltage- and ion-dependent conductances. Currents similar to those commonly seen in ORNs from other species were observed. In addition, two types of responses to odorants were recorded; activation of a non-selective cation current and suppression of voltage activated outward currents. About 25% of the ORNs examined displayed odorant suppression of outward currents, indicating that this was not a nonspecific effect. In cells displaying outward current suppression, injection of current during odorant application resulted in larger membrane depolarization, as would be expected for a reduction in membrane conductance due to suppression. In these cells, application of an odor stimulus at a membrane potential near -70 mV evoked no response, while at more positive potentials the cells were depolarized by the odorant. This effect would amplify any odor induced depolarization generated via activation of a cation conductance. Supported by DC00214.

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CELL TYPE SPECIFIC EXPRESSION OF OLFACTORY-ENRICHED MRNAS IN THE LOBSTER OLFACTORY ORGAN

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We have previously used representational difference analysis to identify mRNAs that are enriched in the olfactory organ of the American lobster, Homarus americanus. Of the twenty-six difference products obtained, six of the sequences had significant similarity to known proteins. In the present study, we examined the expression pattern within the olfactory organ of the six identified genes:(1) an ionotropic receptor,(2) an amine beta-hydroxylase,(3) a tubulin,(4) a calcium binding protein,(5) a serine protease, and (6) an alpha2-macroglobulin. In situ hybridization was performed on 50 micron longitudinal sections prepared from segments (6 annuli in length) of the olfactory organ. Hybridization was done with digoxygenin-labeled sense and antisense RNA probes. Specific labeling by antisense probes was observed in all cases. The ionotropic receptor and the tubulin were detected only in the olfactory receptor neurons. The amine beta-hydroxylase was detected only in outer auxiliary cells. The serine protease was detected only in a novel cell type that encircles the bundle of inner dendrites/inner auxiliary cell processes as it passes through epithelium. We propose the name "collar cells" for these cells. The alpha2-macroglobulin was detected in the collar cells and the receptor neurons. We speculate that these cells belong to non-aesthetasc sensilla. We conclude that these six clones include specific markers for three cell types in the olfactory organ and a potential marker for the core cells of sensory sensilla. Supported by award R01 DC02366.

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PHOSPHORYLATION OF VOLTAGE-GATED ION CHANNELS IN RAT OLFACTORY RECEPTOR NEURONS

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In olfactory receptor neurons (ORNs), ligand-odorant receptor interactions cause G protein-mediated activation of adenylate cyclase and a subsequent increase in concentration of the intracellular messenger cAMP. Odorant-evoked elevation in cAMP is thought to directly activate a cation-selective cyclic nucleotide-gated channel, which causes external Ca2+ influx, leading to membrane depolarization and the generation of action potentials. Our data show that in freshly dissociated rat ORNs, odorant-induced elevation in cAMP also activaties cAMP-dependent protein kinase (PKA), which is then able to phosphorylate various protein targets in the olfactory signal transduction pathway, specifically voltage-gated sodium and calcium channels that might modulate the input/output properties of the sensory neurons. This mechanism could take part in the complex adaptation process in odorant perception. In addition, we found modulation of voltage-gated sodium and calcium currents by 5-hydroxytryptamine and the dopamine D1 receptor agonist SKF 38393. These findings suggest that in situ ORNs might also be the target for efferent modulation by dopaminergic and serotonergic projections.

264 Poster XXXV. Orientation to Chemical Stimuli

HABITAT STRUCTURE, HYDRODYNAMICS, AND CHEMICAL ORIENTATION IN STREAM SYSTEMS

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

JUVENILE HAWAIIAN GOBIID FISH EMPLOY ODOR CUES TO LOCATE FRESHWATER STREAMS FROM THE OCEAN AND TO GUIDE THEM UP THEIR TERMINAL WATERFALLS

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The Hawaiian islands have only 4 species of riverine freshwater fish, all of which are members of the goby family and possess an unusual amphidromous life history. These fish spend their adult lives in the headwaters of streams where they lay eggs in nests. Upon hatching, larvae are swept to sea where they develop for a few months, after which they re-enter streams. Juvenile fish swim great distances inland coming out of the water (using fused fins as a suction cup) to scale the large (100m) waterfalls which characterize these systems. This study asked whether odor cues serve to guide these tiny (1 cm) fish during this journey. Behavioral responses of recently captured juvenile gobies were assayed in 2-choice maze with waterfalls located at the head of each arm. Gobies demonstrated a strong preference to enter and scale flowing stream water when it was tested against seawater. This preference persisted when the former was diluted 100-fold. Stream waters were also strongly preferred over the spring waters from which they originated suggesting that organic compounds released into streams serve as the attractant(s). This was confirmed by tests of conspecific odor (pheromones) which stimulated climbing activity. Tests of different stream waters also found the attractant to be innately recognized and widely distributed. My study is the first to demonstrate that an amphidromous fish uses odor to locate spawning streams, a strategy that appears to make ecological sense given the scale of the aquatic environment and its unpredictability. Funded by the State of Hawai'i.

THE EFFECTS OF ANTENNAL LESIONS ON ORIENTATION BEHAVIOR OF THE CRAYFISH, *ORCONECTES RUSTICUS*

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Numerous animals use chemical cues within their environment to execute various behaviors. One of these behaviors is orientation to a food source. Crayfish, in particular, can orient to food sources under a number of different conditions. It has not been determined, however, if and how these animals with complete or partial impairment of their chemosensory appendages can successfully locate a food source. To determine the role of antennae and antennules in this behavior, the orientation patterns of crayfish with various degrees of antennal lesions were examined. Analysis of the results confirmed that crayfish successfully locate distant food sources through chemotaxis using antennae and antennules. It appears that crayfish use the spatial information from bilateral sampling to successfully orient. Animals using both antennae exhibited increased walking speed and speed to source, decreased heading angles towards the source and decreased average distance from the source than animals with the use of one or neither antenna(e) and antennule(s).

CONDITIONED TASTE AVERSION AND LOCOMOTOR CIRCLING INDUCED BY MAGNETIC FIELDS

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Advances in magnetic resonance imaging have resulted in more powerful machines with higher-strength static magnetic fields. The effects of high-strength magnetic fields on behavior are largely unknown. We have previously shown that exposure in a 9.4T magnetic field is sufficient to induce a conditioned taste aversion (CTA) and induce brainstem expression of c-Fos in rats. To show that behavioral effects from magnetic fields are dependent on field strength, duration of exposure, and orientation with the field, rats were restrained in 7, 9.4 or 14T superconducting magnets for various durations. Effects were assessed by scoring locomotor activity immediately after release from the magnetic field, and by measuring CTA acquisition and duration after pairings of a glucose and saccharin solution (G+S) with magnetic field exposure. Magnetic field exposure at all levels suppressed rearing and induced tight circling behavior in the test cage during the first two minutes following magnet exposure. The direction of the circling was dependent on the rats' orientation within the magnetic field: if exposed head-up, rats circled counter-clockwise; if exposed head-down, rats circled clockwise. CTA was induced after 3 pairings of G+S and 30-min 7T exposure, or after a single pairing of G+S and 1-min 14T exposure, suggesting that magnetic field exposure has graded effects on rat behavior. We hypothesize that exposure in high-strength magnetic fields causes vestibular stimulation resulting in locomotor circling and CTA acquisition. NIDCD03198.

TRACKING STUDIES IN THE AMERICAN LOBSTER: EFFECT OF DISTANCE FROM A LEAKY ODOR SOURCE

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The fine-scale structure of aquatic odor plumes contains directional parameters (pulse height, onset slope, patch distribution) that vary with distance from an odor source. Bilateral antennular chemoreception in the lobster is necessary for detection of such directional cues and for search efficiency within a turbulent odor plume. Input from other sensory organs (antennae or walking legs) may play a role in the initial or final approach towards an odor source. Lobsters were tested in a re-circulating flume (8.0 x 1.8 x 0.45 m) at distances of 4 or 7 m from the source. Stimuli were delivered as a low momentum ground 'leaky' plume. The average flow velocity in the tank was 6.0 cm/s. Orientation paths were videotaped and digitized at 1 Hz. Walking speed and heading angle were used to quantify movement patterns. Far from the source larger heading angles and slower walking speeds were observed in addition to extensive antennae waving. As lobster approached the odor source, their headings improved and walking speeds increased suggesting that a spatial gradient within the plume had been found for tracking. Close to the source, lobsters walked slower and began to grasp for food. We observed no apparent differences in tracking behavior with different starting distances. These results suggest that lobsters may be following an internal plume gradient and may orient more strongly chemotactic and less rheotactic or use odor-gated rheotaxis. Acknowledgement This research was supported bt a grant from the Office of Naval Research to JA.

CATFISH POSSESSING A SMALL PORTION OF A REGENERATED OLFACTORY ORGAN CAN DISCRIMINATE AMINO ACIDS

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Olfactory discrimination was studied in adult brown bullhead catfish (*Ameiurus nebulosus*) during renewal of the olfactory epithelium. It was previously shown that catfish cannot discriminate amino acids without a functioning olfactory system. After bilateral extirpation of the olfactory organs (under MS-222 anesthesia), the organs partially regenerated in 2-3 months. EOG responses evoked by amino acids were of smaller amplitude in the regenerated olfactory organs than in intact organs; however, the relative stimulatory effectiveness of the more stimulatory amino acids with respect to the standard (10-4 M L-alanine), L-methionine and L-norvaline, was equal before and after the regeneration. All other amino acids were relatively more stimulatory in the regenerated than in the intact organ. With the exception of L-isoleucine, the L-valine conditioned intact fish and the re-conditioned fish with regenerated olfactory organs discriminated all non-conditioned amino acids from L-valine. The intact fish conditioned to L-alanine discriminated, with the exception of glycine and L-serine, all the non-conditioned amino acids from L-alanine. The re-conditioned catfish with regenerated olfactory epithelia discriminated all the non-conditioned amino acids, including glycine and L-serine, from L-alanine. In two experimental catfish where regeneration of the olfactory organ was minimal (i.e. no visible olfactory lamellae) and a small EOG response to finger-rinse was observed (and no responses to individual amino acids), discrimination of the conditioned from the non-conditioned amino acids occurred.

ATTRACTION TO MATERNAL ODOR BY JUVENILE CRAYFISH, *ORCONECTES RUSTICUS*

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Chemical odor cues have been found to play an important role in the behavior and ecology of several species of juvenile organisms, including crayfish. In crayfish, cannibalism occurs among juveniles of different broods, therefore chemical signals that can convey maternal status may be important for protection. The aim of this study is to identify the role that maternal odor plays in the life of the juvenile crayfish, *Orconectes rusticus*. Past research has shown that young crayfish are attracted to the chemical odors produced by any ovigerous female that is not necessarily genetically related. Research also demonstrates that crayfish in the third stage of development, when recently separated from their mother, seek aid from her, acquiring protection when confronted by an unfamiliar chemical source. Our study tests whether juvenile crayfish are attracted to maternal odors and whether non-maternal odors are aversive. The third stage juveniles were isolated and tested in groups of three individuals selected from the same brood in a Y-maze containing the paired chemical cues of two adults or various controls. The variable measured was the time spent by each juvenile in both sides of a Y-maze. Our results suggest that an attraction to maternal odor exists for juvenile crayfish. These results indicate that juvenile crayfish may have the ability to discriminate between maternal and non-related female chemical cues.

BOUNDARY-LAYER EFFECT ON THE CHEMICAL SIGNAL MOVEMENT ALONG THE BODY OF A MODEL BROWN BULLHEAD (AMEIURUS NEBULOSUS)

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Flow speed influences the spatial and temporal distribution of a chemical signal. However, a boundary layer exists around the olfactory appendages and can further influence the distribution of chemical signal. The research goal is to quantify how morphology and fluid flow interact to influence the movement of a chemical signal along an organism's body. This research is critical for understanding the interaction between hydrodynamics and sensory biology. Brown bullheads have taste buds along their body that are used to sense chemicals that mediate a variety of behaviors. The chemical signal was measured using IVEC 10 along the body of a model brown bullhead. Several Peak parameters were analyzed as well as a spectral analysis. Differences in signal parameters were found along the body and at different flows. The presence of the model, flow and position on the fish model affects the chemical signal properties and thus influences the type of information the bullhead can perceive. There is an interaction occurring between flow, the presence of model and specific areas on the fish indicating that the morphology of the body affect signal attributes and acts as a sensory filter.

GNRH, MIGRATORY BEHAVIOR AND OLFACTORY SENSITIVITY IN HOMING PACIFIC SALMON

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Seasonal migrations for reproduction are common in many mid-high latitude animals and for many species there are strong links between hormones, reproduction and migration. Salmon are particularly well known for their long distance reproductive migrations wherein they home from oceanic feeding grounds to back to their river of origin to spawn. Prior to their seaward migration, juvenile salmon learn (imprint on) site-specific odors associated with their homestream and later use these retained odor memories to guide the final phases of their homing. Over the last decade we have conducted a series of experiments to examine the influence of maturational hormones such as GnRH on the migratory behavior and sensory acuity of homing Pacific Salmon. Specifically, we conducted a series of experiments in which adult salmon that had returned to the University of Washington hatchery were displaced and challenged to return. We used this displacement protocol to evaluate 1) the effect of displacement date and distance on time to return 2) the relationship between endogenous hormone levels and time to return 3) the effect of experimental manipulation of hormones on time to return. Salmon displaced early in the spawning season returned more slowly than salmon displaced later. Time to return was related to plasma levels of maturational hormones and was decreased by administration of exogenous GnRH analogs. These results, in conjunction with biochemical and physiological measures of olfactory acuity suggest that GnRH may influence both olfactory sensitivity and behavioral motivation to return.

273 Workshop XXXVI. Molecular & Functional Mapping of the Olfactory Bulb

MOLECULAR & FUNCTIONAL MAPPING OF THE OLFACTORY BULB

Finger T.,[1] Restrepo D.,[1] Shepherd G.M.[2] *[1]Dept. Cellular & Structural Biology, Univ. Colorado Health Sci. Ctr., Denver, CO; [2]Section of Neurobiology, FMB236, Yale University Medical School, New Haven, CT*

Participants: M Schaefer, Chiquito Crasto Discussants: Michael Leon, Michael Shipley The olfactory bulb is organized into a mapped representation of the olfactory world. Numerous laboratories have published maps of various features including: axon targeting of olfactory receptor neurons expressing particular odorant receptor genes, distributions of various adhesion molecules and growth factors, and markers of activity such as c-fos, calcium imaging, intrinsic imaging, and 2-deoxyglucose. Because each laboratory utilizes different methods for generating these maps, comparison of data across laboratories is difficult. The intent of this workshop is to introduce a standardized web-based system we propose and to discuss what sort of information should be contained in a public database on the olfactory bulb.

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ODOR ELICITED ACTIVITY PATTERNS IN THE MOUSE OLFACTORY BULB REVEALED BY FMRI

Xu F., Kida I., Hyder F., Greer C.A., Rothman D., Shepherd G.M. *Department of Neurobiology and Neurosurgery, and MRC, Yale Medical School, New Haven, CT*

We have used functional MRI to study activity patterns elicited by odorants in the mouse main olfactory bulb (MOB). The temporal resolutions are 8 and 3 seconds/image for single and multiple slice experiments, respectively, the spatial resolution is 200x200x200 μm. With 2-minute exposure, the fMRI revealed highly activated clusters of glomeruli across the whole OB. Clusters in adjacent slices are contiguous and form activated foci. The two largest foci elicited by iso-amyl acetate (iAA) are located in anteolateral and postiomedial regions of the MOB. With 30-second multiple exposures, the activity patterns are reproducible, although the intensity of activity decreased in the following repeated exposures. We tested iAA and benzyl acetate (BA) which share the same acetate moiety but differ in the aliphatic and aromatic parts. In the same slice, iAA and BA activate glomerular clusters located at the same and different regions of the MOB, reflecting their structural relationship. The effects of odorant concentration and exposure duration on the activity patterns are also being investigated. The lateral and medial activity patterns are in agreement with the projection pattern of the receptor neurons revealed by molecular biology studies. These experiments open the possibility of analyzing fMRI patterns in the MOB in gene-targeted mice. Supported by NIH grants R01 DC03710, R01 DC/NS 03972 and DC00210, Human Brain Project and MURI.

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BEHAVIORAL DISCRIMINATION OF SEX PHEROMONE MIXTURES CORRESPONDS WITH PHYSIOLOGICAL PROFILES OF PROJECTION NEURONS

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Many species of fish release complex, taxa-specific mixtures of steroids. To use these signals for communication, fish should be able to discriminate odor blends. In our laboratory, we have shown that 75% of goldfish (*Carassius auratus*) projection neurons are sex-steroid sensitive. Of those, 30% have distinct responses to sex steroids and 20% are blend detectors. Blends of the sex steroids 17,20βP, 17,20βP-S and AD are especially important because they reflect the ovulatory stage of females that release them. To address how these pheromone mixtures affect behavioral responses, we exposed male goldfish to blends of 2 female pheromone components (17,20βP in combination with either AD or 17,20βP-S) and then to blends of all 3 components. Courtship and aggressive behaviors were recorded before and after pheromone exposure. Courtship behavior increased with exposure to blends of 17,20βP-S and 17,20βP. Blends of 17,20βP-S and AD elicited higher levels of aggression but not courtship behavior. Behavioral responses to 3-component blends did not reflect the results of the 2-component experiment. For example, the blend with the highest AD level elicited high courtship behavior and low aggression. Conversely, the blend with the highest level of 17,20βP-S elicited low courtship and high aggression. In conclusion, responses to multi-component pheromonal mixtures are complex, reflecting physiological properties of projection neurons and suggesting that considerable specialization occurs in vertebrate pheromone signals. Supported by NSF/IBN-9723798.

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SINGLE-UNIT RECORDING DEMONSTRATES THAT PHEROMONES ARE DISCRIMINATED BY A COMBINATION OF LABELED-LINE AND POPULATION CODING IN THE GOLDFISH OLFACTORY BULB

Hanson L.R., Sorensen P.W. *Grad Program in Neuroscience, U of MN, St. Paul, MN*

Although it is known that olfactory bulb (OB) neurons can discriminate between closely-related odorants, how they encode information associated with fundamentally different classes of biologically-relevant odors (such as pheromones) is unclear. To answer this question the chemospecificities of OB projection neurons (PNs) must be understood. We addressed this issue in the goldfish for which 5 odor classes are well characterized: sex steroids (sex pheromone), prostaglandins (another sex pheromone), bile acids (aggregatory pheromone), nucleotides (feeding), and amino acids (feeding). Responses were recorded from 56 PNs to these odor classes, each of which we found to stimulate different receptors using cross-adaptation. Thirty-eight PNs responded consistently to odorants. The majority of these neurons (33) responded to more than 1 odor with excitation or suppression (8 of these were excited by both a pheromone and another odor), but a few (5) responded to just 1 odor. Examining these patterns, we discovered that most PNs (23) responded to 1 of the 5 classes of odors in a distinctive manner and had well-defined spatial distributions (see also Masterman et al., this symposium). While nearly all (37) PNs responded to pheromones in some way (suggesting population coding), all 5 PNs that responded to just 1 odor were pheromone-specific, suggestive of a 'labeled-line'. We hypothesize that a network of PNs in the fish OB uses a combination of population coding and labeled-lines to encode pheromonal odors. NIH/DC03792; NSF/IBN9723798.

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RESPONSE OF MALE *DROSOPHILA* TO FEMALE PHEROMONES

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We have cloned two *Drosophila* genes, *Masc29a* and *Smac42a*, whose remarkably specific expression patterns suggest a role in the perception by males of courtship-modulating pheromones. 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 the detection of female courtship-activating pheromones. While these two genes have no sequence similarity to proteins with known functions or to each other, they are related to two small families of proteins encoded by the *Drosophila* genome. Most, if not all members of these two families are only expressed in male appendages and may therefore also be involved in pheromone response. To directly test whether the cells that express *Smac42a* and the sensilla in which they are present are required for pheromone response, we have generated animals in which those cells are ablated by specific expression of a cell death gene, *Grim*. Elimination of the courtship response of *Drosophila* males to female pheromones requires both the ablation of *Smac42a*-expressing cells and the removal of the olfactory segment of the antennae; either lesion alone has no effect. These findings reveal that males possess two redundant chemosensory systems that detect female courtship-stimulating pheromones: one requires the antennae, whereas the other requires the subset of chemosensory sensilla on the front legs that express *Smac42a*.

278 Symposium XXXVIII. Odor/Taste Interactions and Flavor Perception

INVESTIGATING TASTE AND AROMA INTERACTIONS IN FOODS

Hollowood T.A., DeGroot L., Davidson J.M., Linforth R.S., Taylor A.J. *Food Science, Nottingham University, Sutton Bonington, United Kingdom*

For generations it has been recognised that addition of taste compounds, e.g., sugar, enhances the overall flavour of foods. Despite such anecdotal evidence, interactions between taste and aroma in foods are not well understood. Most foods are a complex, heterogeneous mixture of different organic and inorganic compounds. It is not surprising, therefore, that many studies have shown evidence of physicochemical interactions involving taste and aroma compounds within food. However, the physical and chemical properties of volatile and non-volatile compounds are not always sufficient to explain flavour perception during eating. Opinions on the likelihood of taste/aroma interactions at a perceptual level are mixed. One view is that the two act independently, and that any measure of total flavour intensity is a simple addition of the two parts. Alternatively there may be a perceptual interaction whereby the addition of non- volatile components results in a synergistic effect on the perception of aromas. Evidence to support aroma/flavour interactions was seen in two recent studies. Firstly, a combination of sensory analysis and MS Nose™ has shown that an increase in viscosity does not affect the volatile concentration reaching the nasal receptors despite a decrease in flavour and sweetness. Furthermore, in experiments where taste and aroma were infused into panellists' mouths, retronasal aroma intensity was clearly affected by altering delivery of the tastant. In both experiments, cognitive effects would seem to be responsible as the physicochemical effects were not significant.

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INTERACTION OF THE SENSES IN THE CONSTRUCTION OF FLAVOR

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The interaction among taste and other modalities associated with flavor has been investigated only sporadically. In rats, taste, temperature and touch information converge on neurons in the NST, and this affiliation is maintained throughout the neuraxis. Most thalamic taste cells also respond to temperature, to touch, or to all three. Olfactory and gustatory convergence has been demonstrated at both the NST and PBN. Moreover, taste and visceral afferents interact in the hindbrain, presumably to enable the changes in taste responsiveness that follow physiological manipulations. In primates, there has been no dedicated exploration of multimodal responsiveness in NST or thalamus. In primary taste cortex (IO), a few gustatory neurons also respond to touch, olfactory stimuli, or visual inputs. These responses are in register for quality, i.e., if a cell is activated by glucose, its olfactory sensitivity will be to sweet odors, and its visual response to the sight of the syringe from which glucose is delivered. Taste activity in IO is not influenced by the visceral signals associated with being fed to satiety. In secondary taste cortex (OFC), gustatory, olfactory, and visual afferents converge, and about 30% of the neurons are responsive to two of these modalities. That proportion increases in the amygdala and hypothalamus, where 50% of taste cells also respond to odors, again in register by quality. Satiety also suppresses the responsiveness of these cells. The convergence of both external and viscerosensory afferents onto higher-order neurons may permit the construction of flavor, with its associated hedonic value.

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NEURAL SUBSTRATES OF TASTE/SMELL INTERACTIONS AND FLAVOUR IN THE HUMAN BRAIN

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Taste and smell have separate peripheral substrates and neuroanatomical pathways, however it is their combined sensation that results in flavor perception. Sensation of the texture, temperature, and appearance of food may also alter its flavor, and as such, may be considered additional constituents of flavor perception. A putative "flavor cortex" has been identified in the macaque caudal orbitofrontal cortex. In this region unimodal, bimodal, and multimodal taste, smell, texture, and visual neurons have been described by Rolls and colleagues. Often a single multimodal cell will respond best to corresponding cross-modal qualities, such as the taste of salt and the smell of salmon. It is therefore likely that flavor is represented in this region of the non-human primate brain. Neuroimaging studies in humans have begun to uncover the neural substrates of olfaction and taste in the human brain. However, to date there has been little investigation of the neural substrates of flavor perception. The following questions will be discussed in terms of current literature and future directions for research: 1. Where is flavor represented in the human brain? 2. What are the neural substrates of taste/smell interactions such as mixture suppression and the attribution of taste properties to odors? 3. In terms of the functional neuranatomy, what is the contribution of the gustatory versus the olfactory system to flavour processing?.

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A JUDGMENT MODEL FOR RATINGS OF COMPLEX CHEMOSENSORY STIMULI

Frank R.A. *Psychology, University of Cincinnati, Cincinnati, OH*

It is generally acknowledged that estimates of sensory magnitude are strongly influenced by stimulus context. Recent studies with multi-modal mixtures provide evidence for an influence of response context as well. Response alternatives set a context for observers that influences their approach to a psychophyiscal task prior to the presentation of the stimuli. Observers must develop a strategy for dealing with the task, and the number and type of response alternatives represent constraints that the experimenter imposes on the observer. Studies of odor-induced enhancement of taste ratings provide support for this view. For example, strawberry odor enhances ratings of sweetness for mixtures of sucrose and strawberry odorant when only sweetness ratings are made by observers. Odor-induced enhancement disappears when fruitiness is provided along with sweetness as a response alternative. Response alternatives can also influence descriptions of complex stimuli such as monosodium glutamate. A model for the judgment of complex chemosensory mixtures is described which attempts to account for the influence of both perceptual and conceptual factors on ratings of multidimensional stimuli.

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SUBTHRESHOLD INTEGRATION OF TASTE AND SMELL: THE ROLE OF EXPERIENCE IN FLAVOR INTEGRATION

Breslin P.A., Doolittle N., Dalton P. *Monell Chemical Senses Center, Philadelphia, PA*

Flavor is experienced when tastes, odors, and somatosensations are combined into the experience of the food or beverage that is in the mouth, e.g., apple pie. Recently, we demonstrated that subthreshold tastes and smells integrate. Subjects could reliably detect the presence of an odor presented at ~30% below its detection threshold, if a taste solution was in the oral cavity at ~30% below its respective detection threshold. This effect was not due to the generic integration of all tastes and odors. Rather, benzaldehyde (cherry) integrated nicely with sodium saccharin (sweet) but not with monosodium glutamate (MSG) (savory). We hypothesized that the common experience of sweet-cherry flavor resulted in chronic changes to the CNS components that subserve flavor perception. To test the role of experience, we intuited that the MSG taste would be likely to integrate with odors associated with savory dishes, such as garlic or onions. We determined whether MSG would show subthreshold integration with allyl sulfide (onion/garlic odor), while benzaldehyde (cherry odor) served as the control odor. Although, we confirmed that benzaldehyde does not integrate with MSG, we found mixed support for our hypothesis that allyl sulfide and MSG would show integration; only half the subjects showed integration. We must, therefore, re-evaluate either (1) the role of prior-experience on subthreshold integration of odors and tastes or (2) our assumption that a high percentage of people commonly experience the savory taste of MSG with the odor of garlic and onions. Supported by R29 DC02995 (PASB) and RO1 DC03704 (PD).

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COGNITIVE INFLUENCES ON ODOR/TASTE INTERACTIONS IN MIXTURES

Prescott J. *Sensory Science Research Centre, University of Otago, Dunedin, New Zealand*

Odors that take on taste characteristics, e.g. sweetness, through associative pairing with tastes are also able to enhance the intensity of tastes in solution. However, this effect is sensitive to whether the odor/taste mixture is treated analytically as a set of discrete qualities, or synthetically as a flavor. We examined if these different perceptual strategies would influence whether an odor would initially take on taste characteristics, and its ability to enhance taste intensity. Subjects received multiple exposures to mixtures of sucrose with odors varying in initial familiarity and smelled sweetness, or the odors and sucrose solutions separately. Two groups that received mixtures made intensity ratings that promoted either synthesis or analysis of the odors and taste. A synthetic strategy selectively increased odor sweetness for low familiarity odors. There were increases in the odors' ability to enhance sweetness, but independent of strategy employed, perhaps due to an ineffective analytical strategy. Addressing this, Experiment 2 found that, while odors became sweeter smelling irrespective of group, sweetness enhancement only occurred with a synthetic strategy. This group also showed sweetness enhancement with a "non-exposed" control odor. Experiment 3 showed that this was due to the single co-exposure with sucrose that odors received in the pre-test. Thus, while even a single co-exposure with sucrose is able to produce a sweeter smelling odor, only a synthetic strategy produces an odor that will enhance sweetness. These data show that the integration of odors and tastes into a flavor perception is cognitively mediated.

284 Poster XXXIX. Development of Olfactory Glomeruli

CO-LOCALIZATION OF 5-HT$_{2A}$ RECEPTORS AND β1-ADRENOCEPTORS IN THE MAIN OLFACTORY BULB OF THE RAT

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We have shown previously that norepinephrine (NE) and serotonin (5-HT) interact at the level of the olfactory bulb in neonate rats to facilitate conditioned olfactory learning. NE seems to be critical in the induction of learning whereas 5-HT has a permissive role; while the 5-HT$_2$ receptor agonist DOI promotes sub-threshold activation of β-adrenoceptors (isoproterenol) to induce odor preference learning, a higher dose of isoproterenol can overcome 5-HT fiber depletion of the olfactory bulb to induce learning. The cells that mediate the interactions described above in the bulb are not known. Noradrenergic fibers terminate substantially in the deeper layers of the bulb with only few fibers reaching the glomerular layer. Although βreceptor distribution is clear, the cells receiving the input are not. Serotonergic input terminates in all layers and are especially dense in the glomerular layer. 5-HT$_{2A}$ receptor mRNA and protein have been shown in mitral cells, tufted cells and granule cells by *in situ*hybridization and immunocytochemical studies. To learn the postsynaptic target of the NE-5-HT interaction within the olfactory bulb is important for us to understand the actual physiological and molecular processes underlying the odor preference learning. Our hypothesis that the NE-5-HT interaction occurs in mitral cells was confirmed by the double label immunofluorescent study of rats at different ages with the aid of confocal microscopy.

285 Poster XXXIX. Development of Olfactory Glomeruli

THE OMP-LACZ TRANSGENE MIMICS THE UNUSUAL EXPRESSION PATTERN OF OR-Z6, A NEW ODORANT RECEPTOR GENE: IMPLICATION FOR LOCUS-DEPENDENT GENE-EXPRESSION

Pyrski M.M.,[1] Xu Z.,[1] Walters E.,[2] Gilbert D.J.,[3] Jenkins N.A.,[3] Copeland N.G.,[3] Margolis F.L.[1] *[1]Anatomy & Neurobiology, University of Maryland Baltimore, Sch. of Med., Baltimore, MD; [2]Biochemistry & Molecular Biology, Howard University Med. Coll., Washington, DC; [3]Mouse Cancer Genetics Program, NCI-Frederick, Frederick, MD*

Reporter gene expression in the olfactory epithelium (OE) of H-lacZ6 transgenic mice mimics the expression pattern known for some odorant receptor (OR) genes. The transgene construct in these mice consists of the lacZ coding region, driven by the proximal olfactory marker protein (OMP) gene promoter and shows expression in a subpopulation of olfactory neurons. To address mechanisms underlying the OR-like expression pattern of the lacZ-construct, we analyzed the transgene flanking region and identified OR-Z6, a new OR gene that exhibits the highest sequence similarity (85%) to a human ortholog at a syntenic chromosomal location. We show that the expression patterns of OR-Z6 and the lacZ-transgene bear strong similarities. Expression of both genes is primarily restricted to the same medial subregion of the OE. Axons from both neuronal subpopulations project to the same aspect of the anterior olfactory bulbs. Furthermore, co-localization analyses in H-lacZ6 mice demonstrate that OR-Z6-reactive glomeruli receive axonal input from lacZ-positive neurons as well. These results suggest that the expression of both genes is coordinated and that transgene expression in H-lacZ6 mice is regulated by locus-dependent mechanisms. DCD03112 (FLM), DHHS (NC, NJ, DG).

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CARBOHYDRATES AS AXON GUIDANCE MOLECULES IN THE OLFACTORY SYSTEM

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We are interested in determining the guidance mechanisms for development and regeneration of the olfactory pathway. While odorant receptors are involved in targeting olfactory axons, it may be that cell surface carbohydrates mediate sorting and selective fasciculation of primary olfactory axons en route to glomeruli. If so, axons expressing the same cell surface carbohydrates must target topographically fixed glomeruli in the bulb. We tested this hypothesis by mapping the position of glomeruli innervated by axons expressing NOC-3, a glycoform of N-CAM. NOC-3 axons converged on glomeruli that were consistently located within the same topographical position in the bulb suggesting that these molecules may indeed play a role in axon guidance. Next we generated transgenic mice that express the human blood group A glycosyltransferase under the control of the OMP promoter in order to disrupt cell surface expression of carbohydrates on primary olfactory neurons. These mice are being crossed with P2-tau-LacZ transgenic mice so that the topography of axon can be visualized. Preliminary analysis of one line has revealed that the olfactory bulb of neonates has an enlarged ventricle. In order to address the role of carbohydrates in a damaged and regenerating olfactory system, we performed a series of olfactory bulb ablations in postnatal P2-tau-LacZ mice. After bulbectomy, regenerating P2 axons are able to converge and form glomerulilike loci in the frontal pole. We are currently examining whether axons expressing specific carbohydrates are also able to sort out and target loci following bulbectomy.

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EFFECT OF ANTENNAL GRAFTS BETWEEN TWO MOTH SPECIES ON OLFACTORY PROCESSING OF SEX PHEROMONES

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In many insects mate location is facilitated by chemical signals that, in moths, are processed in the macroglomerular complex (MGC), a subset of sexually dimorphic glomeruli in the antennal lobe. We are using the technique of transplanting larval antennal imaginal disks between males of two moth species, the corn earworm, *Helicoverpa zea*, and the tobacco budworm, *Heliothis virescens*, to test hypotheses on glomerular organization and processing of sex pheromone blends. The sex pheromones of these species share the same major pheromone component but also have important differences involving minor components and behavioral antagonists. Confocal-microscope based studies of interspecific transplants between males revealed that the ingrowing antennal fibers induce the formation of an MGC arrangement characteristic of the donor species. Thus, *H. zea* males with *H. virescens* antennal disks formed an *H. virescens*-like 4-lobed MGC, and *H. virescens* males with *H. zea* antennal disks formed an *H. zea*-like 3-lobed MGC. Electrophysiological tests with individuals of the latter transplant type indicated that central neurons responded to the presence of donor pheromonal components. However, despite sensory axonal and MGC characteristics similar to normal *H. zea* males, transplant individuals preferentially flew upwind to sources of the *H. virescens* blend with some individuals also responding at higher dosages to the donor *H. zea* blend. Supported by NIH 1 R55 DC04443-01.

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IN VITRO ANALYSIS OF INTERACTIONS BETWEEN OLFACTORY RECEPTOR GROWTH CONES AND CENTRALLY DERIVED GLIA

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Axons from olfactory receptor neurons (ORNs) must sort into groupings of similar odor sensitivities in route to their glomerular targets. In the moth *Manduca sexta*, a dramatic reorganization of ORN axons occurs in the antennal nerve in a glia-rich "sorting zone" (SZ) adjacent to the antennal lobe (AL). Experimental reduction of glia prevents the normal sorting and targeting of MfasII-positive ORN axons, implying that glia are key modulators of axonal behavior. The current study examines, *in vitro*, interactions between ORN axons extending from antennal explants and glial-cells isolated from the SZ and the AL. Live-cell imaging using DIC optics has shown that, on a con A/laminin substrate, simple growth cones usually tip ORN neurites. Upon encountering glia, growth cones often elaborate extensively, forming large lamellar processes, and pause for many hours, indicating a change in growth cone adhesive properties. Confocal microscopy of cultures stained for F-actin and α-tubulin has confirmed that growth cones contacting glia adopt complex morphologies, with thickened filapodial arrays and microtubule-based branches. Experiments currently in progress explore the possibility that nitric oxide (NO) is used as an intercellular signal, since in other systems NO has been shown to modulate growth-cone behavior, and in recent *in vivo* studies inhibition of NO synthase lead to defects in AL development. Other experiments are addressing whether changes in free-Ca^{++} levels are required for initiation of the growth cone response.

DEVELOPMENT OF OLFACTORY GLOMERULI:THE ROLE OF EXTRACELLULAR MATRIX MOLECULES

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Olfactory sensory cell axons (OSNs) follow stereotypic spatial and temporal paths in the establishment of the olfactory pathway (Gong and Shipley, 1995; Treloar et al., 1996; Treloar et al., 1999). Extracellular matrix (ECM) molecules have been localized to the developing olfactory pathway and proposed to have a role in establishing the primary olfactory projection (Gong and Shipley, 1996; Treloar et al., 1996). The aim of this study was to determine whether ECM molecules have a role in late embryonic olfactory development when glomeruli (bundles of synaptic neuropil) are formed. We examined the distribution of 4 ECM molecules in the developing mouse olfactory system: Laminin and Perlecan (a heperan sulfate proteoglycan), established neurite outgrowth promoting molecules; as well as Tenascin and Condroitin Sulfate Proteoglycans (CSPGs), established neurite outgrowth inhibitory molecules. At E13 (when axons are restricted to the presumptive olfactory nerve layer) both laminin and HSPG were localized around, and within, the olfactory pathway. CSPGs were highly expressed in the mesenchyme surrounding the OSN axons but were absent within the pathway. Within the developing OB, CSPGs were absent from the marginal zone and present at lower levels within the ventricular zone. Tenascin was not present within the olfactory system at this age. At E17 (when protoglomeruli first emerge) laminin and perlecan were found within the olfactory nerve and glomerular layers. CSPGs were downregulated within the mesenchyme but upregulated in ensheathing glia. Tenascin was highly expressed within the deeper layers of the OB at this age. These data demonstrate that both inhibitory and attractive ECM molecules are present in spatiotemproal positions to affect olfactory pathway development.

BOTH AN EPH-LIKE RECEPTOR TYROSINE KINASE (RTK) AND ITS LIGAND, EPHRIN, ARE EXPRESSED IN THE DEVELOPING OLFACTORY SYSTEM OF THE MOTH, *MANDUCA SEXTA*

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Eph-family RTKs and their ephrin ligands have been shown to play important roles in axon guidance and cell migration during neuronal development in many systems. We are examining the role of Eph receptor/ephrin signaling in the olfactory development of the hawkmoth, *Manduca sexta*, a model organism in which some of the key intercellular interactions have been well documented. We have cloned MsEph, an Eph-like RTK, and MsEphrin, a putative ligand for MsEph. Amino acid sequence of predicted MsEph and MsEphrin proteins are most similar to Eph and ephrin in *Drosophila*, respectively. Northern blot analyses show that both genes are expressed in the brain and antenna at higher levels in early stages during which olfactory receptor axons enter antennal lobe (AL) and synaptogenesis in glomeruli occurs. In situ hybridization shows the presence of MsEph transcripts in a subset of the antennal lobe neurons and in a subset of cells in the sensillar side of antennae. Immunohistochemistry using antisera raised against a peptide near the N-terminus of MsEph reveals that MsEph protein is localized in neuronal processes in both the antenna and AL. These observations suggest that Eph receptor/ephrin signaling may play an important role in the developing olfactory system, possibly in targeting axons of both peripheral and central olfactory neurons. Supproted by Flinn Foundation Predoctoral Award and Charles E. Culpeper Biomedical Pilot Initiative 99-294.

BEHAVIOR OF OLFACTORY RECEPTOR AXONS GROWING NEAR AND IN EXPLANTS OF THE AXON SORTING ZONE OF THE MOTH OLFACTORY SYSTEM

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Receptor neurons (ORNs) with similar receptive properties are distributed across an epithelial region but their axons terminate in a small number of glomeruli, implying that at some point in their trajectory to the CNS, the axons sort into groups of like axons. In the moth *Manduca sexta*, this process occurs in a glia-rich zone of the antennal nerve at the entry to the antennal (olfactory) lobe of the brain. In ongoing experiments, we are comparing the behavior of ORN axons as they navigate near and within tissue from different segments of the primary olfactory pathway. When explants of antenna containing ORN cell bodies are placed in a collagen matrix near, but not contacting, slices of antennal nerve and various brain tissues, sorting zone tissue is not as strongly attractive to ORN axons as are other regions of the brain. When the explants are placed directly adjacent to sorting zone slices and the axons visualized with lipophilic dyes, ORN axons are seen to enter the slices and navigate through the glial domain. Some of the axonal growth cones are extensive and, in fixed preparations, some of the growth cones display flattened regions where the axons approach glial cells, reminiscent of the behavior of ORN growth cones contacting dissociated sorting-zone glial cells, as observed in live-cell recordings (see abstract by Tucker et al.). These results lay the groundwork for perturbation experiments aimed at determining which signaling molecules play roles in axon-glia nteractions in the sorting zone. Support: NS28495, NS20400.

RETRONASAL PERCEPTION AND THE TEMPORAL PROCESSING OF ODORANTS

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Odorants are perceived via orthonasal (nose) or retronasal (mouth) routes. Recently, Jinks and Laing (1999) demonstrated that odorants in mixtures delivered via the nose are often perceived in series, and there is a critical time difference between odorants that determines whether temporal order is discerned. Whether temporal processing occurs during retronasal delivery of odorants is not known. Temporal processing could be induced by the differential adsorption of odorants by the mucus in the pharynx which could act like a chromatographic column and adsorb and desorb odorants on the basis of their water solubility, volatility or molecular weight. Accordingly, the perception of 4 pairs of tasteless equi-intense (retronasal intensity) aliphatic odorants, differing in the latter physicochemical properties within each pair, was investigated using a time-intensity paradigm. Measurement of 'time to maximum intensity', 'maximum intensity' and 'duration to extinction' indicated with 3 of the 4 pairs that the member which had the greater water solubility, higher volatility and lower molecular weight required a shorter time to reach maximum intensity and extinction. Identification of other physicochemical properties that account for the data are currently being investigated. Sponsor: Dr B A Livermore (Member)- Delete I Hutchinson as sponsor Jinks, A. & Laing, D.G. (1999). Cognitive Brain Res. 8, 311-325.

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IDENTIFICATION OF PROTEINS THAT MEDIATE PHEROMONE RECOGNITION IN COCKROACHES

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Pheromones have been studied most extensively in insects. However,the role of pheromone binding proteins (PBPs), the first components of the recipient's olfactory system to interact with pheromones, and the molecular components of the signal transduction and propagation machinery remain still poorly characterized. Cockroaches offer advantages, because their large antennae are easily amenable to physiological and biochemical approaches, and pheromones - and the behavioral responses they elicit - have been well characterized. cDNA libraries were constructed from antennae of sexually mature males and females of the German cockroach (*Blattella germanica*) and the brown-banded cockroach (*Supella longipalpa*) and screened with a male-enriched antennal probe. Thirty-three inserts were sequenced and, as predicted from their high abundance, putative PBPs comprised 15% of the clones. Sequence alignments revealed 6 cysteines at characteristic positions and identified *Drosophila* homologues. Another 15% of the clones encoded membrane receptors and signaling proteins that may function in pheromone reception. Northern blot analyses and *in situ* hybridizations revealed selective expression in male antennae for one of the putative PBPs (Slm44), whereas another PBP (Slm66) is also enriched in male versus female antennae. Phylogenetic analysis showed that Slm44 resembles the *Drosophila* LUSH protein. Studies on *Drosophila* together with high-throughput sequencing studies on the cockroach system are a powerful approach to identify new components that mediate insect pheromone reception.

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ALBUMIN-AN IDEAL PHEROMONE CARRIER PROTEIN

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Proteins are involved in the circuitous transport of (Z)-7-dodecenyl acetate (Z7-12:Ac), the sex pheromone of Asian elephants from its origin in females to its delivery to male vomeronasal sensory cells.Utilizing a radiolabeled photoactivatable analog we identified a 66 kDa protein as the 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. During preovulatory estrus Z7-12:Ac is present in serum in high concentrations, bound to a protein. In emitted urine, Z7-12:Ac is bound to albumin. This protein is remarkably suited for transport and delivery of Z7-12:Ac. The influence of pH on the binding of Z7-12:Ac and albumin affects transport and delivery processes. Increased amounts of Z7-12:Ac are detected in follicular stage serum especially when pH is lowered experimentally or protease is added. During the follicular stage urinary pH in vivo elevates to pH 8.4. In vitro maximal Z7-12:Ac-albumin binding occurs between pH 8-9. Binding experiments demonstrate that in voided urine albumin slowly releases the pheromone, making it available for detection by male elephants for prolonged periods of time. We postulate that upon contact with truncal and/or vomeronasal mucus of considerably lower pH, albumin quickly releases the remaining bound pheromone, creating an intense pulse of the pheromone in the chemosensory organs. Supported by NIH grant RO1-DC03320 (to L.E.L.R).

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RETRONASAL AND ORTHONASAL ODORANT INTERACTIONS: MASKING

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Twenty subjects (median age 20, 11 female) each selected concentrations of anise (A), cinnamon (C), coffee (Co), peppermint (P), and strawberry (S) food-grade liquid extracts for which vapor phase orthonasal (ortho), or retronasal (retro), intensities matched ortho or retro vapor phase intensity of 33% diluted orange (O) extract, and then learned retro, and ortho, veridical names of the matched concentrations and of O standard. In a later session, after retro and ortho identifications (ID) of the 6 odorants (2% errors ortho; 10%, retro), 10 randomized ortho & retro pairs of odorants were given 3 times, with instructions to either initially exhale (retro 1st) or inhale (ortho 1st). Odorant presentation containers were used (Pierce and Halpern, Chem.Senses, 21, 529-543, 1996). RESULTS:*Control pair*: **Co-Co**, Co was an ID on 97% of retro 1st trials & 100% of ortho 1st; Co-Co, 50% of retro 1st ID, 66%, ortho 1st. *Test pair examples*: **P-Co** (retro-ortho loci), P ID = 85% retro 1st & 88% ortho 1st, Co 80% & 78%; P-Co ID, 27% retro 1st & 61% ortho 1st. **Ci-A** (retro-ortho loci), Ci ID = 70% retro 1st & 64% ortho 1st, A 73% & 64%; Ci-A ID, 13% & 39%. **Co-Ci** (retro-ortho loci), Co ID = 85% retro 1st & 85% ortho 1st, Ci ID 60% and 59%; Co-Ci ID 5% & 46%. **Co-S** (retro-ortho loci), Co ID = 77% retro 1st & 86% ortho 1st, S 72% & 64%; Co-S ID 8% & 49%, S-Co 43% and 8%. **P-S** (retro-ortho loci), P ID = 95% & 86%, S 59% and 66%; P-S ID 7% & 53%, S-P 46% and 3%. CONCLUSIONS: Correct ID of dissimilar retro-ortho pair components were < retro or ortho alone; correct ID of pair <<< ID of components. B.C. Sun support: NY Sci. Ed. Prog.

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OLFACTORY SPECIFIC SNMPS OF LEPIDOPTERA DEFINE A UNIQUE FAMILY OF INVERTEBRATE CD36-LIKE RECEPTOR PROTEINS

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SNMP1-Apol (Antheraea polyphemus) is uniquely and abundantly expressed in receptor membranes of olfactory neurons responding to sex-pheromone, expresses coincident with odor response, and is homologous with the CD36 family of membrane-bound receptor proteins, all supporting a central role in odor detection. SNMP1-Apol homologues were identified from Bombyx mori, Heliothis virescens and Manduca sexta; proteins ranged 75-80% identity and are designated SNMP1. A 2nd M. sexta SNMP homologue, previously partially sequenced (Robertson et al., 1999), was fully sequenced and characterized; this protein shared 26-27% identity with the SNMP1s and is designated SNMP2-Msex. SNMP sequences identified 14 Drosophila and 4 C. elegans homologues. Expression of both SNMP1-Msex and SNMP2-Msex is antenna specific, occurs simultaneously with olfactory function, and is localized to olfactory receptor neurons. Findings support SNMPs may play general and central role in odor detection in insects & the CD36 gene family is widely represented among the animal phyla. SNMPs are the only identified neuronal members of the CD36 family and thus expand the activities of this gene family to roles influencing brain function and behavior.

DIFFERENTIAL EXPRESSION OF CLUSTERED OBP GENES: A COMPARATIVE STUDY OF THE DISTRIBUTION OF PBP AND GOBP2 IN MANDUCA SEXTA

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Odorant Binding Proteins (OBPs) are differentially expressed among classes of sensilla which have different odorant specificities. The genomic sequences of two OBPs of M. sexta were sequenced and characterized: Pheromone Binding Protein 1 (PBP1) and General Odorant Binding Protein 2 (GOBP2). Both genes reside together with coding regions separated by about 3000 bp; both genes are oriented in the same direction and are ecdysteroid regulated. Sequences comparisons with other PBPs and two Drosophila OBPs suggest lepidopteran exon units are conserved within Lepidoptera but not between Lepidoptera and Diptera. Neither gene coexpresses in the same sensilla. Both genes express in adult male and female sensory epithelium, within the same sensory field in females but in nonoverlapping sensory fields in males. GOBP2 but not PBP1 expresses in larval antennae and palps; expression is regulated in a manner consistent with ecdysteroid control. These system is a model for studying how two homologous genes are differentially expressed in a manner consistent with divergent sensory neuron phenotypes.

THE IMPACT OF MUCOSAL SOLUBILITY ON CHANGES IN ODORANT PERCEPTUAL INTENSITY

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This study examined the impact that mucosal solubility has on changes in odorant perceptual intensity. Fifteen normosmic subjects, with and without nasal dilators, rated the perceptual intensity of 9 odorants (propionic acid, butyric acid, isopropanol, hexanol, trans-cinnamaldehyde, heptanoic acid, octanol, hexanoic acid, pentanol) at 2 concentrations using the Green Scale. The lower and higher concentrations of each odorant had perceptual intensities of 20 and 40. As expected, when dilated, subjects rated all the odorants as being more intense. The odorants were ranked based on the relative increases in perceptual intensities. To determine the relative mucosal solubility of the 9 odorants, normosmic subjects, while performing velopharyngeal closure, had a continuous stream of odorant injected in one nostril. The stream was collected from the contralateral nostril and sent to a photoionization detector. At equilibrium, the ratio of the number of molecules exiting the nose to the total injected served as a means of ranking the odorants. The rankings of the increases in perceptual intensity and odorant mucosal solubilities were highly correlated. The three odorants that showed the least enhancement in intensity were also the three odorants that had the lowest ratio of molecules sorbed by the mucosa. Likewise, the odorants which showed the greatest enhancement had the highest ratios. Although the perceptual intensity of an odorant is due to many factors, these data support the hypothesis that mucosal solubility, in part, determines how perceptual intensity will change as nasal anatomy changes.

CHARACTERIZATION OF GLUTATHIONINE S-TRANSFERASES EXPRESSION AND ACTIVITY IN THE OLFACTORY EPITHELIUM

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Glutathione S-transferases (GSTs) are phase II biotransformation enzymes that catalyze the conjugation of glutathione to electrophiles. Despite the reported localization of GST expression in rat olfactory mucosa, no study has localized these enzymes in mouse olfactory tissue. We report here that the C57 mouse olfactory mucosa displays intense localization of the GST mu isoenzyme in the dorsal region, specifically in the sustentacular cells and Bowman's glands. Comparatively, GST alpha isoenzyme exhibited a similar expression profile, however the staining was less intense. Immunogold labeling identified significant staining of the GST mu isoenzyme throughout the apical regions of the sustentacular cells. GST mu exhibited the most intense staining compared to the alpha and pi isoenzymes following analysis of Western blots, and all isoenzymes were consistent with reported molecular masses. Our immunohistochemistry and immunoblot analyses indicate that GST mu is the predominantly expressed isoenzyme in mouse olfactory tissue. In addition, there was significant enzymatic activity of the total GST towards 1-chloro-2-4-dinitrobenzene substrate. Our studies suggest a significant contribution of Bowman's glands and sustentacular cells in olfactory function, and provide evidence for a putative role in odorant biotransformation. Supported by HUMAA Endowed Chair Award to EW and the Howard University Specialized Neuroscience Research Program (NIH/NINDS 1 U54NS394004).

THE OLFACTOROME: EXAMINING THE GENE EXPRESSION REPERTOIRE OF OLFACTORY MUCOSA

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We identified a set of genes highly expressed in the olfactory system by constructing and mining a gene expression database that allows us to compare 75 developing and adult mouse tissues for their expression of 8,700 genes on the Incyte Mouse Gem 1 microarray. A single common reference mRNA was used for all arrays, and all tissues were analyzed on duplicate arrays. The adult mouse olfactory mucosa (OM) expressed 105 genes at 2 fold higher than whole mouse reference. Of known genes, highest expressed genes in OM included an uncharacterized palate, lung and nasal epithelium transcript (>32X), paraoxonase 1, lactotransferrin, and protein kinase3. Log2 transformed ratios of the overexpressed genes were subjected to hierarchical tree cluster analysis using Pearson Correlation, and segmented into genes highly expressed in only OM vs. also in one or more other tissues. One cluster was confined to the OM and included sphingosine phosphate lyase, neuronal leucine-rich repeat protein 1, plexin 3, and ten expressed sequence tags (ESTs). Another cluster of genes was detected based on their predominant dual expression in both OM and olfactory bulb (OB). These included eight ESTs, β-amyloid precursor-like protein, and notch 2-like protein. Coordinately-regulated genes were also found in brain, lung, and respiratory mucosa. Identification and classification of genes that define the OM and OB will allow for gene discovery and provide insight into neurogenesis, OM differentiation, and neuronal plasticity.

301 Poster XLI. Receptors and Ligands

MOLECULAR EVOLUTION OF THE INSECT CHEMOSENSORY RECEPTOR SUPERFAMILY

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The insect chemoreceptor superfamily consists of the odorant receptors expressed in the antennae and palps, and the gustatory receptors expressed in the mouthparts and other organs. The odorant receptor family consists of a tightly defined group of 61 genes/proteins, while the gustatory receptors are a far more diverse group of 66 proteins encoded by 58 genes (three alternatively spliced genes encode 2, 4, and 5 proteins). In addition, five nematode genes encode proteins that constitute three phylogenetic lineages within the superfamily, indicating that it predates the arthropod/nematode split. The odorant receptors appear to represent a single lineage within the superfamily that might have evolved along with adaptation to terrestriality. Examination of odorant receptors from other insects supports this antiquity. Analyses of the molecular evolution of these proteins reveals extremely high levels of divergence for their N-termini, with only the C-termini and a final intron placement allowing definitive recognition of members. Intron evolution is extreme, with most gene losing most ancestral introns, and many novel introns being acquired.

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RECONSTITUTION OF OLFACTORY RECEPTORS THAT RECOGNIZE OVERLAPPING SETS OF ODORANTS WITH DIFFERENT SPECIFICITY

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Odor discrimination is likely to be established based on a combinatorial receptor code model in which the identities of different odorants are encoded. In order to obtain experimental evidence of this model, we identified mouse olfactory receptors functionally from single cells that responded to structurally-related odorant molecules by using calcium imaging and single cell RT-PCR techniques. The cloned receptors were functionally expressed in HEK293T cells to decipher relationship between ligand properties and receptor structures. We found that some receptors recognized overlapping sets of odorants with different affinity and specificity. These results provide direct functional evidence to prove the receptor code model, and further demonstrate that a receptor code for an odorant is unique for each odorant, but it changes at different odorant concentrations, consistent with an observation that perceived quality of an odor changes at different concentrations. We also examined the signal transduction cascades activated by the receptors, demonstrating that the receptors coupled to stimulatory G proteins, resulting in cAMP increases upon ligand stimulation in the HEK293T cells. The molecular bases of odor discrimination at a receptor level appear to associate with the mechanism for integrating odorant signals from distinct sets of odorant receptors in the olfactory bulb and ultimately in the olfactory cortex.

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REPERTOIRE OF HUMAN OLFACTORY RECEPTORS

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The identification and cloning of all functional human olfactory receptors (ORs) is an important initial step in understanding receptor specificity and combinatorial encoding in human olfaction. We identified known human ORs by extensive keyword and homology-based search of DNA and protein databases. Novel ORs were identified by reiterative homology searches of translated human genomic sequence databases, particularly the high-throughput genomic sequences, using queries corresponding to the known hORs. Genomic sequences containing areas of significant homology to ORs were searched for open reading frames of sufficient length (>250 aa), which were translated and compared to the known ORs. The criteria for recognizing a particular OR gene product as a full-length receptor were the presence of an ORF starting with ATG codon and a complete seven transmembrane domain. Each sequence was further analyzed to detect structural features deemed incompatible with receptor function; some putative ORs were discarded by this criterion. These intentionally minimalist criteria filtered out most OR pseudogenes. All functional OR candidate genes were cloned from several individuals by genomic PCR. We report the identification and cloning of approximately 350 putative full-length olfactory receptor genes and sequence analysis of the predicted OR gene products. Nomenclature of human olfactory receptors is proposed. We believe that these sequences represent essentially complete repertoire of functional human olfactory receptors.

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FUNCTIONAL ANALYSIS OF A RECOMBINANTLY EXPRESSED HUMAN ODORANT RECEPTOR

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Olfactory receptors constitute a large family of G-protein coupled receptors that are expressed in the olfactory receptor neurons in the nose. They are activated by odor molecules acting as ligands, as the first step of signal transduction in the olfactory pathway leading to the detection and discrimination of odors and the perception of smell. To address the problem of olfactory perception at a molecular level, we have recently cloned, functionally expressed and characterized the first human odorant receptor, OR 17-40, and its specific activating substances: helional and heliotropyl (Wetzel et al., J. Neuroscience, 1999). Here we report the functional expression and the characterization of the receptive range of another member of the cluster of odorant receptor genes located at chromosome 17, OR 17-p110. Application of a mixture of hundred different odorants elicited a transient increase in intracellular calcium at human embryonic kidney (HEK) 293 cells which were transfected with a plasmid containing the OR17-p110 encoding DNA and a membrane import sequence. By subdividing the odorant mixture in smaller groups we could identify one effective component: cyclamal. Testing the activity of more than 20 structurally related molecules we found eight other compounds which could also activate the receptor in a concentration dependent manner. Comparison of these ligands to non-activating odorants in view of individual molecular features, such as alternate functional groups, substitution or length of the carbon chain, provided new insight into the relationship between odorant structure and activity.

FUNCTIONAL EXPRESSION AND CHARACTERIZATION OF A *DROSOPHILA* ODORANT RECEPTOR IN A HETEROLOGOUS CELL SYSTEM

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Odorant receptors (ORs) constitute the molecular basis for the detection of volatile odorous molecules and the perception of smell. Our understanding of chemical senses has been greatly expanded by the discovery of the OR gene families in vertebrates and in the nematode *C. elegans*. Recently, candidate *Drosophila* OR genes have been identified. The putative ORs do not possess any primary sequence identity with known vertebrate or *C. elegans* receptors, but belong to the family of G protein-coupled receptors according to their predicted seven transmembrane topology. In order to prove olfactory function of these proteins, we expressed a member of the putative *Drosophila* OR gene family in *Xenopus laevis* oocytes. Using two-electrode voltage-clamp recording we identified four odors, cyclohexanone, cyclohexanol, benzaldehyde, and benzyl alcohol that activated the receptor at low micro-molar concentration, and structurally related substances that did not. This report shows for the first time the function and specificity of a member of the recently identified familiy of *Drosophila* odorant receptors expressed in a heterologous system.

SEQUENCE HOMOLOGY OF OLFACTORY RECEPTORS CLONED FROM MOUSE GOMERULAR TISSUE PUNCHES

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Olfactory receptor neurons (ORNs) express one or a few olfactory receptors (ORs) and axons from ORNs expressing a given OR converge on a single pair of glomeruli in the main olfactory bulb (MOB). ORNs transport mRNA coding for their respective ORs to these axon terminals in the glomeruli. Three contiguous punches of tissue (A,B,C), 250 um diameter, each encompassing approximately three glomeruli, were removed from 20um sections of the dorsolateral mouse MOB, total RNA was extracted from the punch tissue, and RT/PCR cloning of 380 bp OR cDNA sequences was performed using degenerate primers derived from the mouse OR gene family. One OR each was cloned from punches B and C, and three were cloned from the A punch. These were compared with 120 published mouse OR sequences. Amino acid sequence homologies with these previously cloned ORs ranged from 21% to 47%. Comparisons within the series of punch clones revealed the following homologies: B vs C: 45%; B vs A1: 31%; C vs A1: 34 %. A second series of three punches from a different animal yielded one clone from the B punch and two clones from the C punch. The results indicate that closely positioned glomeruli receive input from ORNs expressing ORs which exhibit a wide range of homology relationships. These analyses will be useful for understanding odorant coding properties of ORs and local interglomerular interactions in MOB. *Supported by grants NIDCD(J.S.K.) and NSF(J.E.M.).

CORRELATION OF ODOUR RECEPTOR ACTIVITIES BY COSMO-RS σ-MOMENTS

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Odour binding activities for 19 organic compounds at 14 odour receptor proteins taken from recent measurements have been correlated with a new class of molecular binding descriptors which result from the COSMO-RS solvation method. These COSMO-RS σ-moments are expected to yield a linear correlation for any logarithmic partition coefficient data. Because binding activities, which can be considered as a kind of partition coefficients, are only known on a crude experimental scale, a special regression technique was used. Reasonable correlations have been found for most receptors. The new approach allows the simple prediction of odour receptor activities of other compounds. In addition the σ-moments introduce the space of odour compounds, and the resulting σ-moment-coefficients introduce a metric for receptors. Thus, similarity of compounds with respect to odour activity can be expressed by a distance in σ-moment space, and similarity of receptors can be expressed as a distance in σ-moment-coefficient space.

ELECTRICAL AND CHEMICAL STIMULATION OF SPECIFIC REGIONS OF THE PBN ELICITS INGESTIVE OROMOTOR BEHAVIORS IN CONSCIOUS RATS

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The central medial (CM) and ventral lateral (VL) subnuclei, as well as the adjoining waist region (W), of the parabrachial nucleus (PBN) contain neurons responsive to gustatory stimuli (Halsell and Travers, '97) and project to brainstem oromotor centers (Karimnamazi and Travers, '98). The role of these areas in oromotor behaviors was investigated by stimulating the PBN in conscious rats and counting oromotor responses (Grill and Norgren, '78). Overall, the application of current (0.4 msec, 50 Hz, 50-200 μA) into CM, VL or W via implanted stainless steel wire electrodes caused a 40-fold increase in ingestive oromotor behaviors ($n=5$, $p<.05$). Stimulation outside the PBN ($n=6$) as well as within the PBN but outside CM, VL and W ($n=3$) did not significantly increase oromotor behaviors over pre-stimulation periods. Microinjection of glutamate (100-400 nl/10 sec, 0.1-100 mM) into CM, VL or W via implanted cannula ($n=8$) caused a 50% increase in ingestive oromotor behaviors over pre-stimulation levels ($p<.05$) and a 400% increase over vehicle injections in the same rats ($p<.05$). In 4 rats, electrical and chemical stimulation of CM, VL or W was performed sequentially via combined electrode-cannula systems (Plastics One). In these rats, oromotor behaviors were increased 100x by electrical stimulation and 7x by glutamate microinjection over pre-stimulation periods ($p<.05$). The data suggest that neurons in the CM, VL and W subnuclei of the PBN are involved in the initiation of ingestive oromotor behaviors in rats. [Support: NSF (IBN 9603184)].

EFFECTS OF GLOSSOPHARYNGEAL NERVE TRANSECTION AND REGENERATION ON QUININE-STIMULATED FOS-LIKE IMMUNOREACTIVITY IN THE PARABRACHIAL NUCLEUS OF THE RAT

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Previously, we demonstrated that regeneration of the glossopharyngeal nerve (GL) is required for the recovery of quinine (Q)-stimulated oromotor rejection behaviors and Fos-like immunoreactivity (FLI) in gustatory portions of the nucleus of the solitary tract (gNST), the first central synaptic relay in the gustatory system (King et al., J. Neuroscience 20:8426-8434). In the current study, we are examining the distribution of FLI in the parabrachial nucleus (PBN), the second obligatory brainstem synapse, of the same animals (N=79). Preliminary data (N=38) indicate that 1)compared with water (W)-stimulated controls, rats intraorally infused with 3 mM Q show significant FLI in the "waist" area of the caudal third of PBN ($p<.01$); 2)a high correspondence exists between the numbers of Q-stimulated FLI-neurons in this area and in subfield 5 (~ medial half of the rostralcentral subdivision) of the gNST; 3)bilateral transection of the GL significantly attenuates the number of Q-, but not W-, stimulated FLI neurons ($p < .01$); 4) this neurotomy-induced effect is enduring (up to 94 days) for rats in which the GL did not regenerate; but, 5)regeneration of the GL after 52 days restores Q-stimulated FLI to control values ($p=.94$). We are currently analyzing other PBN subdivisions and the remaining subjects to explore possible correlations between Q-stimulated FLI-neurons in the PBN and oromotor rejection behaviors. Supported by NIH R01-DC01628.

TASTANT EVOKED ACTIVITY IN THE PRIMARY GUSTATORY NUCLEUS OF GOLDFISH

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The primary gustatory nucleus in the goldfish, the vagal lobe, provides an unique opportunity for understanding central taste processing. The vagal lobes are large, laminated structures that receive primary sensory input from the gustatory nerve roots that innervate the palatal organ and branchial surfaces of the mouth. These structures are highly innervated and utilize both taste and touch to sort potential food items. There is an "orotopic" representation of these surfaces within the vagal lobes so that different oral surfaces are represented in different lamina while different positions within the mouth are represented at different locations across the lobes. In order to address the issue of how taste is encoded in the vagal lobes, we have developed and *in vivo* preparation of the goldfish that allows us to record from neurons in the vagal lobes while stimulating the oral surfaces. Goldfish were immobilized and a respiratory flow of water was provided into the anterior of the mouth. Multiple unit, extracellular recordings from the vagal neurons were then made while tastants were added to the respiratory flow. Our initial findings indicate that the tastants alanine, arginine and quinine activate different units in the vagal lobes. Mechanical stimulation (air bubbles) also activates many units, but due to the large number of units activated in this way it has not yet been determined if mechanical stimulation and tastants activate the same neurons. Supported by NIH grant DC00147 to T. Finger.

CORRELATION BETWEEN THE PLASMA ZINC CONCENTRATION AND THE CHORDA TYMPANI NERVE RESPONSES TO VARIOUS TASTE STIMULI IN ZINC-DEFICIENT RATS

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We previously found that long-term zinc deficiency decreases taste sensitivity at the level of the chorda tympani (CT) nerve in rats. In the present study, we therefore investigated the relationship between the plasma zinc concentration and the taste sensitivity to various stimuli in rats by recording the electrophysiological responses of the CT nerve. Male 4-week-old Sprague-Dawley rats were divided into four groups (Zn-Deficient, Low-Zn, Zn-Sufficient and Pair-fed). After feeding on the experimental diet for 0, 4, 10, 14, 21, 28, 35, and 42 days, the rats were anesthetized, and we recorded the responses of the whole CT nerve to various taste stimuli and to carbonated water. The plasma zinc concentration was measured by atomic absorption spectrophotometry. We confirmed that there is a correlation between the plasma zinc concentration and the CT nerve response to 0.20 M NaCl in Zn-Def rats ($r=0.733$), and we noted a markedly reduced taste sensitivity to 0.20 M NaCl at below 363 ppb of plasma zinc. We also showed a correlation between the plasma zinc concentration and the CT nerve response to 6000 ppm carbonated water in Zn-Def rats ($r=0.628$). However, in the case of other taste stimuli, there were no apparent correlations. Thus, the strongest correlation between the plasma zinc concentration and a CT nerve response in Zn-Def rats was that observed when the taste stimulus was 0.20 M NaCl.

FOS REACTIVITY TO MSG AND SUCROSE IN SOLITARY NUCLEUS OF THE RAT

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Spatially distinct areas of the gustatory portion of the nucleus of the solitary tract (gNST) are activated by quinine and sucrose, as demonstrated by Fos-like immunoreactivity (FLI) (Harrer & Travers, *Brain Res.,* 1996, *711*:125-137; King et al, *J. Neurosci.,* 1999, *19*:3107-3121). This suggests a spatial organization to the responsiveness of gNST to different gustatory stimuli. Conditioned taste aversion studies with rats suggest that monosodium glutamate (MSG) and sucrose share common taste characteristics when amiloride is added to reduce the Na+ component of MSG taste (Stapleton et al., *Chem. Senses,* 1999, *24*:449-457). Initial studies in this lab have also shown both overlapping and spatially distinct patterns of FLI cells in gNST induced by MSG and sucrose (both mixed with amiloride). Rats stimulated with MSG expressed increased FLI in the medial region of the gNST. Sucrose ingestion resulted in FLI expression in medial and lateral aspects of the gNST. FLI was more intense in the caudal portion than in the rostral portion of gNST in both MSG and sucrose stimulated rats (Stapleton et al, *Soc. Neurosci. Abs.,* 2000, *26*:1975). To further specify the topography of taste specific cells within gNST to sucrose and MSG, FLI following unilateral nerve transections of chorda tympani and glossopharyngeal nerve are being studied. Preliminary results indicate a diminishment in FLI cells in gNST ipsilateral to the nerve transection consistent with taste specific topography. [Supported by NSF Grant IBN-9982913 to ERD].

313 Poster XLII. Taste Pathways

ALDOSTERONE ATTENUATES NACL RESPONSES IN RATS WITH UNILATERAL CHORDA TYMPANI SECTION

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Previous research has shown unilateral transection of the chorda tympani nerve combined with dietary sodium restriction results in an attenuated response of the intact chorda tympani to sodium salts in adult rats. Specifically, 4 - 8 days after transection, the relative sodium response of the contralateral chorda tympani is reduced compared to controls. The mechanisms involved in this paradigm are still unclear. Aldosterone, a hormone important to sodium homeostasis, is elevated during times of sodium depletion. Therefore, to assess the possible role of aldosterone as a mediator in this phenomenon, rats on a sodium replete diet were implanted with mini-osmotic pumps containing either saline or aldosterone. In addition, some of the aldosterone treated rats received unilateral chorda tympani transection immediately before implantation of the osmotic pump. After 4 – 8 days, whole chorda tympani nerve responses to 50-500 mM NaCl, NaAc, KCl, and NH_4Cl were obtained. Results indicate that aldosterone treated, nerve transected rats had a significantly lower relative response to sodium salts than control groups. There was no apparent affect of aldosterone alone. These results implicate the rise in circulating aldosterone in sodium deprived rats as a key factor in the reduced sodium response in rats with unilateral chorda tympani transection. Supported by NIH grant DC00407.

314 Poster XLII. Taste Pathways

TEMPORAL PATTERN OF LICK-CONTINGENT ELECTRICAL STIMULATION IN THE NUCLEUS OF THE SOLITARY TRACT PREDICTS BEHAVIORAL REJECTION

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Previous work from our lab has shown that electrical stimulation of the nucleus of the solitary tract (NTS) can produce a taste-like sensation in the awake, behaving animal. Results showed that rats could acquire an aversion to lick-contingent electrical stimulation in the NTS designed to mimic the temporal pattern of the electrophysiological response to sucrose (sucrose simulation pattern). In the present experiment, we tested the acceptability, measured by the number of licks, of lick-contingent electrical stimulation designed to mimic the electrophysiological response to quinine (quinine simulation) in rats that received no prior behavioral training. Results showed that all animals avoided licking when it was paired with the quinine simulation pattern but resumed licking when it was paired with the sucrose simulation pattern. When exposed to a pulse train of evenly spaced pulses at the same frequency as the quinine simulation pattern or to a pulse train where the interpulse intervals of the quinine simulation pattern were randomized, rats drank significantly more than they did with the quinine simulation pattern. Histological examination of brain tissue revealed that stimulating electrodes were located in the NTS only in animals that rejected the quinine simulation pattern. These observations suggest the temporal pattern of a taste responses in the NTS may be an important part of the neural code and that it may contribute to an animal's decision to accept or reject a taste stimulus. Supported by NSF grant BNS-0077965 to P.M.D.

315 Poster XLII. Taste Pathways

EFFECTS OF GLOSSOPHARYNGEAL ANESTHESIA ON TASTE RESPONSES IN THE NTS OF THE RAT

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Previous studies have provided evidence for an inhibitory interaction between the facial and glossopharyngeal (GL) nerves. Specifically, anesthetization of the chorda tympani (CT) nerve has been shown to produce increases in the neural responses to quinine in the nucleus of the solitary tract (NTS) in hamsters. Psychophysical studies in humans have also reported that CT anesthesia causes an increase in the perception of bitter-tasting stimuli, when applied to the contralateral tongue. To further investigate this interaction, neural responses to gustatory stimuli in the rat NTS were recorded prior, during and post anesthetization of the GL. Preliminary data have shown that GL anesthesia attenuates taste responses in the NTS in a stimulus-specific fashion.

316 Poster XLIII. Transduction in Sensory Cells

POPULATION OF CHEMORECEPTORS AND CHEMOSENSITIVITY IN ADULT RED SEA BREAM *PAGRUS MAJOR*

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To further understand the correlation between chemoreceptors population and olfactory sensitivity, I have begun to investigate the olfactory epithelium of red sea bream by scanning electron microscopy and tested the sensitivity of the olfactory organ by tapping the bioelectrical activity as evoked by amino acids at the olfactory bulb. Ultrastructural observation revealed that the olfactory epithelium is comprised of 3 cell types; sustentacular, olfactory receptor neurons (ORN) and basal cells. Ciliated (CRN) and microvillar olfactory receptor neurons (MRN) were the two bipolar sensory neurons as discerned by their characteristic apical dendrites of cilia and microvilli with the axon-like processes. The somata of the CRN and MRN were located at the basal 2/3 and superficial 1/3 of the neuroepithelial regions respectively. Moreover, a new receptor neuron (MCN) was discovered. The dendritic process of the MCN was buried in the neuroepithelial sheet, exposing its distinctive short micro cilia through a stoma. The mean density/mm ^{2} of the ORNs was 65000 (±11000SD) while the mean sensory area of a lamella was 1.71 (±0.35mm^{2}SD). A 2.05cm^2 of sensory area yields ca 26million ORNS per fish nose. Field potentials were evaluated by a time-series analysis via a FFT. Highly stimulatory amino acids produced a threshold between 10^{-8}-10^{-7}M. Taken together, the large number of ORNs coupled with submicromolar thresholds of amino acids indicates a dominance of odor-guided behavior in the red sea bream making it a complex macrosmatic vertebrate. Acknowledgements: I am indebted to Ms. Ikuyo Tomisako for making this presentation a reality.

RECEPTOR AND SYNERGISTIC MECHANISMS FOR UMAMI AND SWEET SUBSTANCES IN WISTAR RATS

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Umami substances such as MPG and IMP elicit a unique taste called "umami" in humans. Recently, Chaudhari *et al.* (1996) found that the metabotropic glutamate receptor, mGluR4, was expressed in lingual tissues only with taste buds by using PCR method in rats. They also showed that MSG and an agonist for mGluR4, L-AP4, elicited a similar taste in the behavioural experiment. In spite of these findings, it is known that the rats trained to avoid umami substances cannot discriminate the taste between umami and sweet substances (Yamamoto *et al.* 1991). In the present study, therefore, we measured the chorda tympani nerve responses to umami, sweet substances and their mixtures in rats to investigate receptor and synergistic mechanisms for umami and sweet substances. Results are as follows: (1) L-AP4 (5mM) showed synergistic effects like MPG when mixed with 0.01M IMP. (2) The responses to the mixtures of L-AP4 + sweet substances were also synergistically enhanced. (3) Any antagonists for glutamate receptors did not suppress the responses to the mixtures of L-AP4 + IMP and L-AP4 + sweet substances. (4) Gurmarin (50 μM), an anti-sweet peptide, suppressed the responses to the mixtures of L-AP4 + IMP, but it did not suppressed the responses to the mixtures of L-AP4 + sweet substances. These results suggest that umami receptors may not be simply understood by the established glutamate receptors, such as mGluR4, and there are more than two types of mechanisms for eliciting the synergistic effect of umami taste in rats.

RELATIVE EXPRESSION OF DELAYED RECTIFYING K^+ CHANNEL SUBTYPES DIFFERS IN THE THREE TYPES OF LINGUAL TASTE BUDS

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In taste cells, delayed rectifying K^+ (DRK) channels contribute to action potentials and serve as targets for modulation by taste stimuli, either directly or indirectly. Specifically, we have shown that DRK channels are inhibited directly by fatty acids and that dietary fat preferences may be partially due to differential expression of DRK channels. We have used semi-quantitative PCR to estimate the types and relative amounts of DRK channels in taste buds from the fungiform, foliate and vallate papillae. We have designed primers for 9 DRK channels, including members from the *Shaker* (Kv1), *Shab* (Kv2) and *Shaw* (Kv3) families, and compared expression of the mRNA for these channels with actin mRNA in the linear phase of the PCR reaction. PCR products were analyzed on agarose gels and quantified by densitometry. In all three taste buds types, we found expression of RNA for several types of DRK channels. Interestingly, different patterns of DRK expression were noted across taste bud types. The vallate and foliate showed similar expression patterns, with highest expression of Kv1.1, Kv2.1, Kv2.2 and Kv3.1 and lowest expression of Kv1.2, Kv1.5 and Kv3.2. In contrast, fungiform taste buds exhibited high levels of expression of Kv1.5 and Kv3.2, and low or no detectable expression of Kv1.1 and Kv2.1. Differences in the anterior and posterior tongue may affect both the electrophysiological responses of taste cells as well as their chemosensitivity. *Support was provided by NIH DK55809 & Novartis Pharmaceutical Corp. (TAG).*

GLUTAMATE RECEPTOR AGONISTS AND RAT CT RESPONSES

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Monosodium glutamate (MSG) mixed in amiloride may share behavioral properties with sucrose in rats. However, rat multi-fiber chorda tympani (CT) responses to binary mixtures of sucrose and MSG mixed in amiloride are additive suggesting activation of distinct classes of CT neurons (Stapleton et al. (2000) Chem Senses). To characterize CT glutamate sensitivity given the recently cloned mGluR4 rat taste receptor (Chaudhari et al. (2000) Nat Neurosci), we recorded single-fiber responses from 15 CT strands, each containing 1-4 functional fibers, from 7 rats. Neural responses, archived on VCR tape, are being analyzed offline with PC software (RC Electronics) to identify single-fibers by uniform spike amplitude and waveform. To assess glutamate sensitivity, three stimulus sets were applied to the anterior tongue. One set (100mM NaCl, 100mM NH_4Cl, 300mM sucrose) were standard taste stimuli. Another set (50mM MSG alone, 0.5mM inosine 5'-monophosphate (IMP) alone, & MSG-IMP mixture) tested MSG-IMP enhancement. The third set (100mM MSG, 100mM monopotassium glutamate (MPG), 30mM N-methyl D-aspartate (NMDA), 10mM L-2-amino-4-phosphonobutyrate (L-AP4), MSG in .03mM amiloride and NaCl in amiloride) tested agonists for ionotropic (NMDA) and metabotropic (L-AP4) glutamate receptors. MPG and the amiloride-MSG mixture were used to assess responses attributable to glutamate without sodium. Responses to sucrose, L-AP4, NMDA and enhanced responses to the MSG-IMP mixture have been recorded in some strands. We are currently quantifying response profiles of constituent single fibers for presentation. (Support: NIH P01 DC-03013).

IMMUNOHISTOCHEMICAL LOCALIZATION OF ICTACALCIN AND CALBINDIN IN CATFISH OLFACTORY EPITHELIUM

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Evidence suggests that calcium plays a critical role in olfactory signal transduction mechanisms and is believed to influence odor adaptation. Calcium can down regulate cyclic nucleotide-gated (CNG) ion channels in olfactory epithelia, however the precise mechanism by which calcium works is unknown. Some studies suggest that calmodulin or another unknown calcium binding protein may mediate calcium-induced modulation of the CNG channel. To understand the potential role of calcium binding proteins in olfactory mechanisms, the immunohistochemical localization of two calcium binding proteins, ictacalcin and calbindin, was examined in olfactory epithelium of the channel catfish, Ictalurus Punctatus. Ictacalcin, a novel calcium binding protein expressed only in chemosensory tissue of the catfish, is localized to the support cells of the olfactory epithelium and is present in support cells of both the respiratory and sensory epithelium. Calbindin, a calcium binding protein present in a wide variety of species and tissues, is present only in the sensory epithelium and not in the respiratory epithelium. Calbindin staining is present in neuronal cells of the olfactory epithelium, and is limited to a sub-population of neurons. The results indicate differential expression of calcium binding proteins by cells of the catfish olfactory epithelium. Considering the potential role of calcium and calcium binding proteins in odor adaptation, future studies will explore the possible roles of these calcium binding proteins in olfactory mechanisms. Supported by NIH grant R03-DC03246.

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF TASTE CELLS EXPRESSING GUSTDUCIN

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It has been well established that gustducin is an alpha subunit of a heterotrimeric G protein that is expressed in a subset of taste cells (McLaughlin et al., 1992). Previous studies have shown that gustducin is involved in both bitter and sweet taste transduction (Wong et al., 1996), but its precise function has remained elusive. *In vitro* biochemical assays have shown that gustducin activates phosphodiesterase(PDE)(Hoon et al., 1995; Ruiz-Avila et al., 1995), causing a decrease in intracellular cAMP, but the ion channel targets have not been identified. We have begun whole cell patch clamp studies on taste cells from transgenic mice in which the gustducin promoter drives expression of green fluorescent protein(GFP), allowing the gustducin-expressing taste cells to be identified for physiological recording. General comparisons of GFP-labeled and unlabeled cells revealed few differences in basic membrane properties: both types of cells have voltage-gated Na+ and K+ currents, although the Na+ current of some GFP-labeled cells was either small or absent. When 8-bromo cAMP was applied to the bath, 10% of GFP-labeled cells showed a decrease in the voltage-gated K+ current, and/or a decrease in holding current and membrane conductance. Further studies will be required to determine if these responses are linked to taste transduction. Supported by NIH grants DC00766 and DC00244 to SCK.

CARBONIC ANHYDRASE INHIBITOR, MK-927, MODULATES TASTE NERVE FIBER RESPONSES TO BASIC TASTES AND CO2 IN SD RATS

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We previously demonstrated that the integrated responses of the whole chorda tympani (CT) nerve to carbonated water, Quinine-HCl (Q), and L-glutamic acid (Glu) are decreased by the topical application of MK-927, a membrane-permeable carbonic anhydrase inhibitor (CAI) to the tongue surface. The present study was undertaken to reveal which types of CT nerve fibers are affected by CAI treatment. Adult female SD rats were used for single-fiber recordings from the CT nerve. Single-fiber analysis was undertaken using Spike Isolation and Histogram software for Windows 98 computers. After responses to taste stimuli had been recorded, 0.2 ml of 50 mM MK-927 solution was applied to the tongue for 2 min, then the tongue was gently rinsed with deionized water. Soon after, the series of stimuli was repeated. The percentage inhibition or enhancement due to MK-927 of evoked spike activity was then calculated. Those fibers sensitive to HCl or sucrose were not affected by MK-927 treatment, whereas fibers sensitive to Q and Glu showed significantly decreased firing. In the case of NaCl-sensitive fibers, there was no change in the spike activity of N-type fibers, whereas that of the E-type fibers was decreased by MK-927 treatment. The present study suggests that carbonic anhydrase activity in taste-bud cells plays an important role in normal taste sensation, not only for carbonated water but also for certain basic tastes.

MODULATION OF BASOLATERAL NA^+/H^+ EXCHANGE ACTIVITY IN POLARIZED TASTE RECEPTOR CELLS BY PH, CA^{2+} AND CAMP

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A Na^+/H^+ exchange activity (NHE) is present in the basolateral membrane of TRCs. To investigate the basolateral NHE in acid sensing, intracellular pH (pH_i) was monitored by imaging in polarized fungiform taste receptor cells (TRCs) using the fluoroprobe, BCECF. Apical and basolateral sides of the papilla were perfused independently with HEPES-buffered media (pH 7.4; 22° ± 1°C). A decrease in the lingual surface pH from 7.4 to 3.0 gave TRC acidification without pH compensation, i.e. during acid stimulation NHE activity is inhibited. A decrease in basolateral pH from 7.4 to 6.7 also gave sustained changes in TRC pH_i. The NH_4Cl pre-pulse technique was used to measure the rate of pH_i recovery at different set pH_i values. The rate of pH_i recovery decreased with intracellular acidification induced by either apical or basolateral pH_o changes. A decrease in pH_i could inhibit NHE activity via second messengers. Addition of 250 µM CPT-cAMP to the basolateral side decreased resting TRC pH_i and inhibited the rate of pH_i recovery from an NH_4Cl pre-pulse by 55%. Addition of 20 µM ionomycin, a Ca^{2+}-ionophore, alkalinized resting pH_i and increased the rate of pH_i recovery from an NH_4Cl pre-pulse by 400%. The alkalinization induced by ionomycin was blocked in the presence of basolateral MIA, a blocker of NHE. We conclude that during acid stimulation sustained changes in TRC pH_i are due to inhibition of basolateral NHE activity. This could involve an increase in intracellular cAMP or a decrease in intracellular Ca^{2+} concentration. Supported by NIDCD grants DC-00122 and DC-02422.

CALCIUM REGULATED IONIC CHANNELS IN CHEMOSENSORY NEURONS OF A FAMOUS SOIL NEMATODE

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We previously described single channel and whole cell currents present in two chemosensory neurons of the soil nematode Caenorhabditis elegans. These channels may provide the ionic basis for chemosensory transduction and regulation of transmitter release in these non-spiking neurons. We have now studied the regulation of ionic channels in AWA by internal calcium. Excised patches were exposed to either 0 µM Ca^{2+} (calcium chelated by 5 mM EGTA) or to 100 µM Ca2+. Of the channels we previously described, the 'inward flickerer' channels appear to be little affected by increased intracellular Ca^{2+}. The activation potential of the common outward rectifier K^+ channels is shifted to more negative potentials. The high-calcium exposure also exposed large-conductance channels which had been rare or absent in low-calcium conditions. An outwardly rectifying channel of about 120 pS conductance with a negative reversal potential may be one of the *Slo* channels reported to be present in nerve ring neurons by Salkoff. The properties of the native channel are similar to properties of the gene product expressed in a heterologous expression system. A second large conductance channel cannot be associated with any reported *C. elegans* channel. This channel has a unitary conductance of about 200 pS, a reversal potential near 0 mV, and is active at potentials more negative than 0 mV. Under the ionic conditions of these experiments, a reversal potential near 0 mV is most compatible with a non-selective cation channel. This work was supported by NIH grant R01 DC04203.

KNOCKOUT MOUSE MODELS FOR THE STUDY OF TASTE SIGNAL TRANSDUCTION

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We have identified several components of the signaling pathways involved in transducing responses to sweet and/or bitter compounds. These include: 1) α-gustducin, a G protein α-subunit, 2) Gγ13, the γ-subunit of gustducin, 3) α-transducin another taste cell-expressed G protein α-subunit, and 4) TrpT a novel calcium channel expressed selectively in taste receptor cells. Knockout mice provide a powerful tool for elucidating the function of novel genes. In addition to the α-gustducin knockouts, which have been central to demonstrating the key role that this αsubunit plays in bitter and sweet signal transduction, we have produced knockout mice for α-transducin, Gγ13 and TrpT. We have used gene targeting in ES cells followed by microinjection into blastocysts to generate mice lacking the entire coding region of Gγ13. These heterozygous mice are being intercrossed to obtain homozygotes, which will be used for behavioral and electrophysiological studies. Similarly, by gene targeting we have produced ES cells lacking part of TrpT. These ES cells have been microinjected into blastocysts to obtain chimeras. α-Transducin knockout mice in which the region coding for amino acids 64-206 was deleted and replaced with a PGK-Neo cassette were generated. These α-transducin knockout mice have been crossed with α-gustducin knockout mice to produce α-transducin/α-gustducin double knockouts. Initial results of behavioral experiments with knockout mice lacking α-gustducin, or α-transducin, or both G protein α-subunits will be presented.

PHARMACOLOGICAL PROFILE OF TASTE-MGLUR4 DETERMINED BY EXPRESSION AND CALCIUM IMAGING IN HEK293 CELLS

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Taste-mGluR4 is a candidate receptor for umami. This receptor is a novel variant of mGluR4, a metabotropic glutamate receptor found in the brain. In taste-mGluR4, the first half of the extracellular N-terminus, which includes the high-affinity binding site for glutamate, is lacking. When expressed in cell culture, taste-mGluR4 responds to glutamate with a threshold near 100 μM, close to the threshold for detection in intact rodents. Nevertheless, it is unclear whether taste-mGluR4 uniquely underlies umami taste or whether other receptors in taste buds also may be important. To test whether the ligand sensitivity and selectivity of taste-mGluR4 correlates with umami taste, we have expressed taste-mGluR4 with either a promiscuous Gα15 or a chimeric Gαq/i in HEK293 cells and used fura2 to measure calcium responses to applied stimuli. We find that cells expressing taste-mGluR4 (and the Gα) respond to glutamate with increases in [Ca]i. Responses to glutamate and L-AP4, an umami ligand, were concentration dependent and required concentrations similar to those eliciting umami taste. The maximum Ca responses in cells expressing taste-mGluR4 and brain-mGluR4 were not noticably different. However, responses to glutamate were markedly lower than the responses of the same cells to ATP, which stimulates endogenous purinergic receptors. Responses to additional mGluR agonists and antagonists are currently being measured and will allow us to determine whether the pharmacological properties of taste-mGluR4 are compatible with a central role for detecting umami in rats. Supported by NIDCD (DC03013).

OMP-DIRECTED UBIQUITOUS EXPRESSION OF AN ODORANT RECEPTOR IN MOUSE OLFACTORY NEURONS

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The presence of a large repertoire of odorant receptor genes in the mouse contributes to ligand recognition. The expression of individual receptors in only a small fraction of the sensory neurons increases the effort required to characterize the relationship between odorant stimuli and physiological responses. We have generated a mouse line, UbI7, in which a particular odorant receptor, mouse OR I7, is specifically and ubiquitously expressed in all olfactory neurons under the control of the olfactory marker protein (OMP) promoter. The ubiquitous expression of the I7 RNA was demonstrated by in situ hybridization. Northern blot analysis showed that I7 mRNA was expressed in olfactory epithelium at levels 1/10 that of OMP. In situ hybridization also revealed that the expression pattern of endogenous odorant receptors in this animal was retained. The axon convergence and targeting of the OR P2-expressing neurons is apparently not affected. An electro-olfactogram recording showed that the I7-specific odorants heptanal and octanal evoked a dramatically increased response in olfactory epithelium comparing to wild-type animals, indicating that the expressed I7 receptor is functional. Such an animal could be a useful model for studying the function, biochemistry and gene expression of odorant receptors. Moreover, the availability of this genetic system may assist detailed examination of the physiology of olfactory neurons, sensory processing in the olfactory bulb and odorant perception.

ODOR DETECTION THRESHOLDS IN MICE WITH UBIQUITOUS OLFACTORY EXPRESSION OF THE OR I7 RECEPTOR

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In the UbI7 strain, the mouse OR I7 is specifically and ubiquitously expressed in all olfactory neurons under the control of the olfactory marker protein (OMP) promoter. Because of particularly large EOG amplitudes in response to heptanal (7-al) and octanal (8-al), we assessed absolute detection threshold for cineole (C), octyl aldehyde (O) and heptaldehyde (H) in 4 I7 and 9 control (wild-type and heterogeneous) adult mice. Odors were presented in a multi-channel liquid dilution olfactometer. Thresholds were determined using descending concentration steps of ½ log unit (200 trials/concentration/session) and detection failure was defined as performance accuracy of <75% in 400 trials. The lowest concentration with >70% accuracy defined detection threshold. Mean threshold (% of vapor saturation) over all mice for C, O, and H odors were 6.6x10-7%, 1.4x10-6% and 1.1x10-5%, respectively. Differences between I7 and control mice were not significant for threshold values or errors to criterion on any odor concentration. There were also no differences between groups in discriminating hepaldehyde from 1-hexanol and octyl aldehyde from 1-octanol. We suggest that other, higher affinity receptors for 7-al and 8-al are responsible for detection at threshold or that mice are unable to process this unusual sensory information into an appropriate behavioral response.

PARALLEL ANTENNULAR CHEMOSENSORY PATHWAYS FOR ODOR-MEDIATED BEHAVIORS IN THE SPINY LOBSTER *PANULIRUS ARGUS*

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Crustaceans rely on their chemical senses for many important behaviors. The antennules of decapod crustaceans are covered with thousands of chemosensilla (cuticular extensions of the exoskeleton that are innervated by dendrites of chemosensory neurons (CNs)) and are considered their primary chemosensory organ. Most studies on chemoreception in decapods have focused on the prominent aesthetasc sensilla. CNs of aesthetascs project to the glomerular olfactory lobes (OL). However, studies on the lobster's capacity to perform odor-mediated tasks following selective sensillar ablation have revealed that non-aesthetasc antennular chemosensilla are sufficient for many odor-mediated behaviors. CNs of non-aesthetasc sensilla project to the non-glomerular lateral antennular neuropils (LAN). The aim of this study was to identify non-aesthetasc chemosensilla on the antennules of the spiny lobster. We used electron microscopy and electrophysiology to determine the modalities of antennular sensilla. We identified two types of non-aesthetasc antennular chemosensilla - hooded sensilla and simple sensilla. Our results suggest that they are bimodal (chemo- and mechanoreceptive), are present on virtually all segments of the antennules, respond to waterborne chemicals, have CNs with different response specificities, and are present on several lobster species. These bimodal sensilla are expected to mediate antennular chemoreception through an LAN pathway that parallels and has redundancy with the aesthetasc OL pathway. Supported by NSF IBN 0077474 and NIH DC00312.

DISCRIMINATING ODORS THROUGH SYNCHRONY IN GLOMERULAR CIRCUITS: NEW DATA FROM MULTICHANNEL NEURAL-ENSEMBLE RECORDINGS

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Imaging studies show that different odors evoke distinct spatiotemporal patterns of activity across arrays of olfactory glomeruli, but little is known about the specific neural interactions that underly these activity patterns. To address this issue, we used a 3-pronged silicon multielectrode array to record ensemble responses to odors across multiple glomeruli in the moth antennal lobe. Simultaneous recordings from up to 25 neurons revealed a number of reproducible network characteristics. First, different odors evoked distinctly different patterns of spike activity across a given ensemble. Second, different output neurons innervating the same glomerulus always showed similar tuning features, but they often displayed different firing dynamics: "fast spiking" neurons showed graded responses over a broad range of odor concentrations, the responses of "moderate spiking" neurons were relatively independent of concentration, and "irregular spiking" neurons often failed to respond to consecutive stimulus pulses. The most striking observation was that different odors synchronized distinct subsets of neurons reproducibly from one odor pulse to the next. Furthermore, synchrony was not oscillatory, but linked to stimulus dynamics. Ensemble recordings thus revealed precise temporal relationships among specific subpopulations of neurons encoding a specific odor. Synchronized output from one or more glomeruli may serve to strengthen the distributed representation of a given odor across the glomerular array.

MODULAR REPRESENTATIONS OF AROMATIC ODORANTS IN THE RAT OLFACTORY BULB

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Simple aliphatic odorants activate unique combinations of glomerular modules in the olfactory bulb. Each module is comprised of a number of adjacent glomeruli and responds to an odorant molecular feature such as a functional group or an element of hydrocarbon structure. Because aromatic odorants have little conformational flexibility and unique hydrocarbon structures compared to aliphatic molecules, we asked whether they would be coded differently from aliphatic odorants. We exposed rats to aromatic odorants containing aldehyde, alcohol, and methoxy functional groups. We then mapped the evoked uptake of [^{14}C]2-deoxyglucose across the entire glomerular layer. Each of the aromatic molecules that we tested (4-hydroxybenzaldehyde, benzaldehyde, o- and p-anisaldehyde, guaiacol, and vanillin) evoked a unique pattern of modular glomerular activity, much like the aliphatic odorants that we have described previously. Indeed, many of the modules overlapped directly with those responding to aliphatic aldehydes and alcohols. However, the details of the representations differed from the aliphatic odorants of similar carbon number in that the aromatics did not evoke activity in the posterior modules that appear to encode information concerning hydrocarbon structure in aliphatic molecules. We will integrate this new information regarding the spatial representations of aromatic molecules with data characterizing the representations of over 40 aliphatic odorants in order to provide a more generalized model of olfactory coding. This work was supported by grant DC03545 from NIDCD.

OLFACTORY RECEPTOR DISTRIBUTION AND OLFACTORY BULB RECEPTIVE FIELDS IN THE SALAMANDER

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Both spatial and temporal mechanisms likely play roles in how odorants are encoded by the olfactory system. In order to assemble information that can be directly compared from a single experimental species, we and others have studied these mechanisms in an animal model, the tiger salamander, from which numerous anatomical, physiological, biochemical, behavioral, and, most recently, molecular biological data have been gathered. In this analysis, we focused on how spatially distributed events related to individual and populations of epithelial and bulbar neurons interact with one another. We compared odorant receptive fields defined for bulbar mitral/tufted neurons using single unit recording (Kauer and Moulton, 1974) and those defined for ensembles of bulbar neurons using voltage-sensitive dye imaging (Cinelli et al, 1995a,b,c), to epithelial in situ hybridization patterns defined using probes for single salamander olfactory receptor gene messages (Marchand et al, submitted). These comparisons have made it possible to delineate populations of sensory neurons expressing an odorant receptor that were within, verses those that were outside of, receptive fields related to particular odorant responses. These studies have also suggested how patterns of bulbar activity, patterns of epithelial stimulation, and distributions of molecular receptors may be concatenated to generate the spatial attributes of the odorant coding process that have been observed. Supported by grants from NIDCD, ONR, and DARPA (J.S.K.) and NSF (J.E.M).

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GENOMIC ANALYSIS OF THE MOUSE CHROMOSOME-6 VNO RECEPTOR GENE CLUSTER REVEALS COMMON PROMOTER MOTIFS AND A HISTORY OF LOCAL DUPLICATION

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The mouse vomeronasal organ (VNO) is involved in chemosensory detection of pheromones. This responsiveness is mediated through at least two families of G-protein-coupled receptors. The V1R family is estimated to consist of approximately 100 genes. We generated approximately 200 kb of genomic sequence encompassing a portion of the large V1R gene cluster on mouse chromosome 6. In addition to the previously identified VN2 and VN12 genes, six novel V1R genes were identified, including 3 pseudogenes. Four duplications of OR-containing genomic blocks and extensive L1 repeat activity are evident in the region. These genomic events took place as recently as mouse-rat divergence, consistent with the hypothesis that speciation barriers may emerge as a result of repeat-mediated changes in VNO receptor repertoires. We have identified empirical transcription start sites for the functional V1R genes. Two shared ~1-kb patches of non-coding sequence homology are identified, one encompassing putative promoter regions. Examination of the human syntenic region at chromosome 2p13 indicates that this major mouse cluster has been dispersed or deleted in humans. A wider search of the human sequence databases for putative orthologous V1R relationships reveals a widely scattered assortment of pseudogenes. These data suggest that there has been a loss of VNO receptor functional orthology in primate evolution.

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FROM FUNCTIONAL GENOMICS TO FUNCTION OF THE GENOME IN OLFACTORY NEURON DEVELOPMENT

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Suppression subtraction PCR was performed on mouse olfactory epithelium (OE) isolated at the peak of neuronal cell death (maximal TUNEL-positivity, 36-48 hrs post bulbectomy), steady state adult (60d), and the peak of neurogenesis (peak# BrDU+ cells, 6-7 days post-bulbectomy). 18 different mRNAs were identified which were highly induced (>10-fold) as dividing OE progenitors initiated olfactory neuron gene expression. Three of these sequences corresponded to ESTs from genes implicated in the same developmental gene regulation event – methylation-dependent gene silencing and chromatin remodelling. Methyl DNA-binding protein 2 (MBD2) is more highly expressed in the OE than elsewhere in the nervous system. When MBD2 binds to differentially methylated DNA, it recruits Histone Deacetylases(HDAC), which induce conformational changes in chromatin to silence genes in adjacent regions of the genome. HDAC2 is induced in immature olfactory neuroblasts prior to terminal differentiation. We also identified a putative de novo DNA methyltransferase (DNMT) exclusively expressed in the olfactory epithelium after e12 of development. In the developing nervous system, MBD2 is expressed most highly in olfactory and vomeronasal neurons. It forms a complex containing HDAC 2 in differentiating clonally-derived olfactory neurons from the olfactory placode. Inhibition of MBD2 or HDAC function has a significant effect on olfactory neuron development in vitro (by inhibition) and in vivo (by knockout analysis).

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IMMUNOCYTOCHEMICAL AND MOLECULAR ANALYSIS OF OLFACTORY PROGENITOR CELLS IN VITRO

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The olfactory neuroepithelium is unique in maintaining a progenitor population which continues to proliferate robustly and generate neurones even in the adult and it provides an accessible system for studying neurogenesis. We have developed a novel method of isolating putative neuronal progenitor cells from neonatal rat olfactory tissue. In primary culture they generate large multicellular aggregates resembling the neurospheres which are an in vitro characteristic of neural progenitor cells isolated from the CNS. Olfactory turbinates were collected from neonatal Wistar rats, progenitor cells isolated using a method of enzymatic digestion and selective filtration and grown in dissociated primary culture. Neurospheres formed and immunocytochemical analysis showed cells in the spheres expressed nestin, the intermediate filament protein widely used as marker of proliferating neural stem cells, and also GBC-1, a marker of globose basal cells (Goldstein & Schwob, 1996). Interestingly, cells in some spheres also labelled with SUS-4 an antibody marker for sustentacular cells. We were able to promote proliferation of cells in the neurospheres and differentiation of their progeny down the neuronal pathway by treatment with different exogenous growth factors. RT-PCR analysis of individual spheres has allowed us to follow the dynamic alteration of various gene transcripts on treatment with specific trophic factors. Supported by the Garnett Passe & Rodney Williams Memorial Foundation and the NHMRC of Australia.

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POSTEMBRYONIC PROLIFERATION OF OLFACTORY RECEPTOR NEURONS IN SPINY LOBSTERS IS MODULATED BY CENTRAL AND PERIPHERAL FACTORS

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Olfactory receptor neurons (ORNs) proliferate continuously in spiny lobsters, enabling receptor cell addition to accommodate increasing body size, and turnover of existing cells. A further feature of the olfactory system in these animals is the capacity for complete regeneration following injury. This is important because lobster ORNs are located distally in the antennules, which extend from the head and are easily damaged. *In vivo* pulse labeling using bromodeoxyuridine (BrdU) shows that proliferation of ORNs and associated glia normally occurs at one specific site (zone) in the olfactory organ. These cells appear to be derived from precursors located in the antennular epithelium immediately proximal to this zone. Here we describe central and peripheral factors that affect the spatial and temporal pattern of ORN proliferation. Central factors linked to the molt cycle, such as steroid hormones, positively regulate the size and shape of the normal proliferation zone. Local factors associated with peripheral injury also modulate the size and shape of this zone. In addition, local factors play a role in intiating new proliferation zones following accute injuries, which enables regeneration. As in many animals, postembryonic development of lobster ORNs is regulated by both central and local signalling mechanisms. Current studies utilize both *in vivo* and *in vitro* methods and aim to identify trophic factors that regulate normal development of ORNs and regeneration following injury. Supported by NIH DC00312 and NSF IBN0077474.

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PROGENITORS OF GNRH CELLS LIE OUTSIDE THE OLFACTORY PLACODE

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Our lab is studying the development of the olfactory placode and the different cell types, namely olfactory neurons and neuroendocrine cells containing gonadotropin-releasing hormone (GnRH), arising from the differentiating olfactory organ. Our previous work has shown that the olfactory placode develops from a large field of cells at the edge of the neural plate, and the olfactory placode does not appear to be the source of the GnRH neuroendocrine cells of the terminal nerve and CNS. These striking findings have led us to propose a new model for olfactory placode development and search for alternate origins of the neuroendocrine cells thought to arise from the olfactory placode. Using DiI, we have started to label small groups of cells in the neural plate in regions both posterior (cranial neural crest) and anterior (anterior pituitary) to the olfactory fields. Our data indicate that the GnRH cells of the terminal nerve arise from cranial neural crest and migrate anteriorly as the olfactory placode is formed. Cells labeled in the anterior end of the neural plate are found in the anterior pituitary as well as in the migratory route between the olfactory organ and hypothalamus. Finally, we have examined the you-too mutant, that is missing the pituitary, for the presence of the GnRH. In this mutant background the GnRH cells of the terminal nerve are present but those lying in the migratory route between the olfactory organ and hypothalamus are missing. Therefore, we propose that cranial neural crest and anterior pituitary are sources of the GnRH cells thought to arise from the olfactory placode.

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X-INACTIVATION OF THE OCNC1 CHANNEL GENE REVEALS A ROLE FOR ACTIVITY-DEPENDENT COMPETITION IN THE OLFACTORY SYSTEM

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The organization of nervous system is often dependent on neuronal activity and competition between cells. In olfaction, the olfactory cyclic nucleotide-gated channel subunit 1 (OCNC1) is essential for odorant-evoked activity and the X-linked OCNC1 gene is subject to random X-chromosome inactivation. We have generated reporter-tagged OCNC1-deficient mice that permit a direct visualization of OCNC1-deficient olfactory neurons and their projections. Male mice, in which all of the neurons are phenotypically mutant, retain the ability to generate a structurally normal olfactory epithelium and bulb. In heterozygous females, X-inactivation creates a mosaic with two populations of genetically distinct neurons. OCNC1-deficient neurons are slowly and specifically depleted from the olfactory epithelium and display unusual patterns of projection to the olfactory bulb. Remarkably, this depletion is dependent on odorant exposure and is reversed by odorant deprivation. These results suggest that odorants and the activity they evoke are critical for neuronal survival in a competitive environment and implicate evoked activity in the organization and maintenance of the olfactory system. This general approach can be used to create animal models with mosaic tissues consisting of genetically distinct cell types in which one can examine the role of specific gene products in cellular competition.

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REMOVAL OF THE INFERIOR THIRD OF THE SUPERIOR TURBINATE IS NOT ASSOCIATED WITH A SIGNIFICANT DECREASE IN HUMAN OLFACTORY ABILITY

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Objective: Lanza and Bolger have described an endoscopic approach to the sphenoid sinus that involves resection of the inferior portion of the superior turbinate. This study will determine if olfactory tissue can be found in the superior turbinate mucosa and what effect it's removal has on the patient's olfactory ability. **Methods:** The inferior one-third of the superior turbinate was removed from patients during endoscopic sphenoidotomy. This tissue was stained with olfactory marker protein (OMP) antibody. Specimens were graded for content of olfactory epithelium and subepithelial neuronal elements. All patients underwent uni-nasal 12-item smell ID testing before surgery and between 4 and 29 weeks after surgery. **Results:** The histology of 38 superior turbinate biopsies stained with OMP antibody demonstrates that 68% contain no olfactory mucosa, 22% show olfactory mucosa in <25% of the sample, 6% in 25-75% of the sample, and 4% in >75% of the sample. 76% of the superior turbinate samples have no identifiable neuronal elements in the subepithelial tissue, 18% have elements in <25% of the tissue, 3% in 25-75% of the tissue, and 3% in >75% of the tissue. When comparing the pre and postoperative smell test results, 54% of the nostrils have no more than a 1 item change, 43% of the nostrils have a >1 item improvement, and only 3% have a >1 item loss. **Conclusions:** Olfactory tissue is sparse in the inferior portion of the superior turbinate mucosa. Removal of this mucosa during endoscopic sphenoidotomy is not associated with a significant decrease in olfactory ability.

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PEROXYNITRITE-MEDIATED OXIDATIVE STRESS IN OLFACTORY BULBS FROM PATIENTS WITH ALZHEIMER'S DISEASE

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Peroxynitrite, a neurotoxin formed by the interaction of superoxide anion radicals and nitric oxide, reacts with protein tyrosine residues to form 3-nitrotyrosine (NT), an indicator of oxidative stress. We previously demonstrated that olfactory receptor neurons in autopsy tissue from AD patients are more intensely immunoreactive for NT than are those in most control subjects (ISOT 2000). Using an alcohol sniff test, we have confirmed that in the population of volunteers from whom our autopsy tissues are obtained, AD patients are significantly impaired in their ability to detect 70% isopropanol compared to NDE subjects. We have investigated the presence of immunoreactivity for NT and for CD68, a marker for microglia, which are a source of nitric oxide, in OBs from AD and NDE subjects. In AD patients, intense NT immunoreactivity occurred throughout the OB, with the strongest staining in the granule cell layer, and in the endothelium and immediate vicinity of capillaries. Large numbers of microglia occurred in the OBs of AD patients. In NDE subjects, there was a relatively low level of NT immunoreactivity throughout the OB, and noticeably fewer microglia were present. Thus, our results suggest that peroxynitrite-mediated oxidative stress occurs in the OBs of AD patients to a greater extent than in age-matched controls. Support: NIH NIA 1 R01 AG16345 (MLG). The Alzheimer's Disease Research Center, which provides human nasal mucosa and neuropathological/neuropsychological subject data, is supported by NIH P50-AG05144.

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OLFACTORY FUNCTION IN HIV-POSITIVE SUBJECTS

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Chemosensory complaints in HIV-infected subjects have been reported in earlier studies. The aim of the present study was to re-investigate the published data and to evaluate the olfactory performance in HIV-infected patients. Olfactory thresholds (T), odor discrimination (D), and identification (I) was assessed using the "Sniffin' Sticks"-test battery. Seventy-four HIV-positive patients were tested. According to CDC-criteria 38 subjects were classified stage A, 10 stage B, and 26 stage C. None of the subjects exhibited severe cognitive impairement. Compared to normative data all patients performed normally in odor identification (Mean/SEM = 13.5/0.2; 95% Confidence Interval = 13,1-13,9 /normal 50th percentile = 14) and discrimination (12.7/0.2; 95% CI = 12,3-13,1 /norm. 50th perc. = 13) tasks whereas thresholds (6.0/0.2; 95% CI = 5.6-6.4 /norm. 50th perc. = 8.3) were well below the median of normal population. No significant differences in the TDI-scores (F[2,71] = 0.45, p = 0.64) between patients of stage A, B, or C could be observed. This may be interpreted such that olfactory dysfunction is among the primary deficits in HIV-infection which occurs independently of disease stage. Psychometric measurement correlated negatively with the patient`s ability to discriminate odors (r74 = -0,31, p = 0,008). The results of the present study confirm previous work that odor thresholds are elevated early in HIV-infection whereas a decline in identification and discrimination is correlated with reduced cognitive abilities.

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SHIFT OF AMINO ACIDS: A POSSIBLE EXPLANATION FOR SMELL DYSFUNCTION IN CIRRHOTIC PATIENTS

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Aminoacids play an important role in olfaction. One of the most important stimulating neurotransmitters in the olfactory system is glutamic acid. We determined the smell function of 63 patients with cirrhosis of the liver as well as the serum concentrations of all aminoacids and taurin. The majority of the patients exhibited olfactory loss (68%), only 32% were found to be normosmic. Dependent on the degree of liver injury we noticed a statistically significant increase of asparagic acid, glutamic acid and methionine, whereas glutamine was reduced. The serum concentration of glutamic acid was increased up to ten times. The concentration of ammonia increases heavily because of the liver damage. Apart from the effect of ammonia on the brain metabolism, it changes the permeability of the blood-brain-barrier and leads to an increasing up-take of several aminoacids. As the increasing amounts of ammonia cannot be reduced within the urea cycle in the brain, ammonia is reduced in another way. α-ketoglutarate is converted into glutamate by reductive amination and then further converted into glutamine by ATP-dependent amidation. Glutamate and glutamine are continousely produced and secreted into the blood. Due to our hypothesis the elevated concentrations of glutamate might overstimulate the transduction in the olfactory nerves, in the mitral and tufted cells. Therefore the shift of this aminoacid might cause smell dysfunctions.

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OLFACTORY FUNCTION CORRELATES WITH DOPAMINE TRANSPORTER UPTAKE WITHIN THE BASAL GANGLIA IN PARKINSON'S DISEASE AND MULTIPLE SYSTEM ATROPHY

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This pilot study evaluated the relationship between olfactory function and dopamine transporter specific uptake values (SUVs) within the basal ganglia of 16 patients with Parkinsons disease (PD) and 7 with Multiple System Atrophy (MSA). SPECT scans were acquired 3-4 hours after a 740 MBq (20 mCi) IV injection of [Tc-99m]TRODAT-1. During the uptake period, the Smell Threshold Test™ (STT), the UPSIT, and various neuropsychological tests were administered. The olfactory, but not the neuropsychological, measures discriminated between the PD and MSA patients, with the UPSIT showing the strongest discrimination. In this small sample, SUVs were greater in MSA than in PD in most basal ganglia regions sampled. Correlations between the olfactory test scores and SUVs were present within most of the regions evaluated within each of the two diseases. In MSA, for example, the r between STT scores and the whole striatum SUV was -0.92. The analogous r in PD was -0.69. These data suggest the possibility that the differences observed in olfactory function between PD and MSA may relate to differences in dopaminergic activity within the basal ganglia. Whether dopamine-related activity is a predictor of smell function in other diseases or in normal individuals is not yet known. Supported by Grants RO1 AG17524 (DM, PI) and RO1 DC 04278, RO1 DC 02974, and RO1 AG 27496 (RLD, PI) from the National Institutes of Health, Bethesda, MD USA.

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ARE NONTASTERS AT RISK FOR CARDIOVASCULAR DISEASE (CVD)?

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We examined if genetic variation in taste was associated with CVD risk through influencing fat intake and serum lipids. Bitterness of 6-n-propylthiouracil (PROP) and fungiform papillae density are markers for this genetic variation; individuals at the extremes of both may be the most genetically diverse. We classified adults (mean=27±4.6 years) by PROP tasting (PROP ratio; Bartoshuk et al, 1994) and fungiform papillae density (average number per 28mm^2 area on either side of the anterior tongue) into 16 nontasters (5 F, 11 M) with a PROP ratio <0.4 and papillae density <25 and 17 supertasters (12 F, 5 M) with a ratio 1.2 and density >25. Fat intake was assessed by yearly intake of 3 high-fat food groups (high-fat protein, added fat and desserts) and percent calories from total and saturated fat (5, 24-hour food records). Serum from fasting subjects was analyzed for total cholesterol, HDL- and LDL-subfractions. Variables were tested for average and/or distribution differences (p<.05). Females were most likely to be supertasters. Nontasters reported more frequent intake of high-fat protein foods and added fat and consumed greater total and saturated fat calories than did supertasters. Nontasters also had a lipid profile associated with CVD risk: higher total and LDL-cholesterol, and higher total to HDL-cholesterol ratio. These data extend findings of Lucchina et al, 1995: nontasters may have a greater CVD risk through fat intake and serum lipid profile than do supertasters. (NRICGP/USDA 9603745, NIH DC00283 and NIH RR00125 supported).

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TASTE AND OLFACTION IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM

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The prevalence of primary hyperparathyroidism (PHPT) in Austria amounts to 0.08% of the entire population. So together with diabetes mellitus and diseases of the thyroid gland, PHPT is one of the most common endocrine diseases. In all of these patients one or more adenomas of the parathyroid glands can be found. Laboratory blood tests typically find a hypercalcemia and an elevated level of parathyroid hormone. From April 2000 on we tested 50 patients for their ability to smell and taste. Psychophysical testing of olfactory function was performed by means of the "Sniffin' Sticks". The taste solutions were prepared in 8 dilutions in 50% steps starting from 300mg/mL for sucrose, 60mg/mL citric acid, 80mg/mL sodium chloride, and 20mg/mL caffeine. As the dendritic release of fast neurotransmitters relies on N and P/Q-type calcium channels and the magnitude of dendrodendritic transmission is directly proportional to dendritic calcium influx, we were interested whether the elevated calcium in the serum would change the olfactory and gustatory acuity. Our investigation revealed an impairment of both smell and taste. Regarding taste the patient-group performed significantly worse in all 4 taste-qualities. The role of calcium is still not completely understood, but there seems to be evidence that the concentration of the extracellular calcium may influence taste and smell perception.

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STUDIES OF OLFACTORY FUNCTION IN PARKINSON'S PATIENTS RECEIVING ELECTROTHERAPY FOR CONTROL OF EXTRAPYRAMIDAL SYMPTOMS

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Smell loss is an early sign of idiopathic Parkinson's disease (PD). Such loss is unrelated to disease duration, disease stage, use of antiparkinson medications, or to the degree of motoric symptoms. In the last few years, non-pharmacologic options for controlling extrapyramidal aspects of PD have become available, including implantation of devices for electrically stimulating such central brain structures as the subthalamic nucleus (STN). Electrical stimulation of this nucleus can achieve antiparkinsonian effects similar to those produced using levodopa. Indeed, such stimulation typically ameliorates motor fluctuations and dyskineases, and improves most parkinsonian signs. In the present program, we are assessing odor detection sensitivity, odor identification, and short-term odor memory in patients with subthalamic implants during "on" and "off" periods of high-frequency activation. Preliminary data suggest that some measures of olfactory function may be mildly improved in selected patients during the "on" periods relative to "off" periods. The testing of more subjects is needed to verify these observations and to establish subject factors reponsible for or correlated with such improvement.

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EFFECT OF DENTAL DEAFFERENTATION ON GUSTATORY SENSITIVITY

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An epidemiological study was undertaken in order to evaluate taste impairment after dental deafferentation (DD). 196 Subjects were healthy volunteers without history of complication following dental treatments. Localized electrogustometric thresholds (EGM) were evaluated for each subject on 9 tongue loci as well as on the soft palate. 33 Ss without DD constituted a control group. 129 non-smoking Ss who were not receiving any medication were classified according to the number of DD and results showed that EGM thresholds increased depending on the number of DD. Ss with 1 or 2 posterior DD exhibited no significantly different thresholds compared to controls. Ss with 3 or 4 posterior DD exhibited increased thresholds at posterior loci (92% increase, $p = 0.03$, Student t). Ss with 7 or more posterior DD exhibited higher thresholds for posterior loci (208% and 134%, $p<0.001$, $p<0.01$). Subjects with 1 or 2 anterior and 0 to 6 posterior DD exhibited significantly increased thresholds at posterior tongue loci only, whereas subjects with 1 to 6 anterior and 7 to 16 posterior DD exhibited significantly higher thresholds both at posterior and anterior loci. In conclusion, not only the number but also the DD localization was important for the localized tongue sensitivity impairment. Taste thresholds seemed to be locally increased when a topographically corresponding dental branch of the Vth nerve was injured. Gustatory and somato-sensory functional convergence in central relays might be responsible for such a functional intermodality interaction.

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EXPRESSION OF NEURONAL GAP JUNCTION SUBUNIT CONNEXIN 36 IN THE OLFACTORY SYSTEM

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Gap junctional channels, made up of connexin subunits, mediate chemical communication between adjacent cells. Connexin 36 is a gap junction subunit expressed in neurons that may be involved in modulation of neuronal activity. We show that mRNA encoding for connexin 36 is heterogeneously distributed in the olfactory sensory epithelium. The distribution pattern is partially overlapping with that for connexin 43 or 45 (Zhang and Restrepo 1999, Zhang et al. 2000). Within the olfactory epithelium, the hybridization signal is relatively strong in many cell bodies laying between the basal lamina and $^3/_4$ of the way to the apical process, suggesting expression of connexin 36 in olfactory neurons. The hybridization signal is also observed in the periglomerular cells, mitral cells, granule cells and possibly tufted cells in the olfactory bulb. Immunohistochemical studies reveal punctuate immunolabeling in the olfactory epithelium. The immunoreactivity is very strong in olfactory nerve bundles under the olfactory epithelium and in glomeruli of the olfactory bulb. The intensity of immunolabeling in individual glomeruli shows regional variation. Glomeruli displaying high immunoreactive intensity are predominantly distributed in the anterior and posterior regions of the olfactory bulb and posterior regions of the accessory olfactory bulb. Our data suggest that connexin 36 is heterogeneously expressed in the olfactory neurons, which implicates a potential role of connexins in modulating olfactory transduction. Supported by NIH grant DC00244 and DC00566.

ESTIMATING THE NUMBER OF GLOMERULI IN THE HAMSTER MAIN OLFACTORY BULB WITH THE PHYSICAL FRACTIONATOR

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The physical fractionator, a stereological tool for counting objects that extend across pairs of tissue sections, was used to make unbiased estimates of the total number of glomeruli in the main olfactory bulb (MOB) of the adult male hamster. It combines a physical disector probe with fractionator sampling techniques. Cryostat sections (25 μm) processed for synapsin IIa immunoreactivity and counterstained with hematoxylin were used to visualize glomerular profiles. Glomeruli were counted when a profile evident in a reference section was not seen in a paired look-up section spaced 50 μm caudally. Separate counts were tallied in each quadrant, using boundaries established in previous tract-tracing studies (Schoenfeld and Macrides, 1984; Schoenfeld et al., 1985) and confirmed in recent observations on NADPH diaphorase staining patterns (Schoenfeld and Knott, 2001). Preliminary estimates put the total number of glomeruli in each MOB at about 4000. This is twice the number estimated for the mouse MOB (Royet et al., 1988), but is expected given the comparable diameter of glomerular profiles in the two species (85 μm) and the larger size of the hamster MOB. The proportion of this total in each half is comparable. Since 50% of olfactory receptor neurons with knobs (ORNs) project to the medial half vs. 50% laterally, there is comparable convergence onto the average glomerulus in each half. However, since 25% of ORNs project to the dorsal half of the MOB vs. 75% ventrally (Schoenfeld, 2000), there is far greater convergence of ORNs onto the average glomerulus of the ventral MOB. Supported by NIH grant DC3835.

A PREPARATION OF THE PATHWAY OF OLFACTORY RECEPTOR AXONS FOR DYNAMIC IMAGING

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The organization of the olfactory system in the larval sea lamprey allows for the spatial analysis of the pathway of olfactory receptor neurons (ORNs), as the olfactory epithelium, olfactory nerve and olfactory bulb are located in the same horizontal plane. This arrangement allows for viewing serotonergic non-olfactory fibers that extend from the nasal cavity, along the olfactory nerve, and into the olfactory bulb (Zielinski et al 2000 J. Comp. Neurol. 420:324-334). This preparation provides a model for investigating "cross-talk" between non-olfactory fibers and ORNs. For the present study, the ORNs were loaded with the fluorescent calcium indicator Calcium Green 1-Dextran to monitor temporal and spatial changes in calcium levels upon stimulation. We used a protocol adapted from Friedrich and Korsching (1997 Neuron 18:737-752) to load the dye. The pathway of the ORNs was exposed for viewing by vibratome sectioning, then analyzed by confocal microscopy. The axons of ORNs projected into distinct glomerular modules located in specific dorsal, anterior, lateral, medial and ventral positions. The sites of serotonergic innervation, revealed by immunofluorescence following dye loading, provided information regarding putative locations for modulatory interaction between the non-olfactory fibers and the ORNs These results provide a basis for experiments that will examine the spatial pattern of neuronal modulation of afferent olfactory activity. Supported by NSERC grant 46383.

GUANYLYL CYCLASE-D GENE ORGANIZATION AND UPSTREAM REGULATORY ELEMENTS

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Expression of membrane receptor guanylyl cyclase-D (GC-D) is restricted to dendritic cilia of a small population of olfactory sensory neurons that project to a group of atypical glomeruli in the olfactory bulb including the modified glomerular complex (Fülle et al., 1995; Juilfs et al., 1997). These so-called 'necklace' glomeruli are thought to process olfactory cues associated with suckling behavior in neonatal rodents. Coexpression studies suggested that GC-D may function as a chemosensory receptor in a unique cGMP-signaling pathway. Previously, we reported the chromosomal localization and overall genomic organization of the mouse GC-D gene (*Gucy2d*; Yang et al., 1996). To better understand *Gucy2d* expression and function, we now isolated mouse BAC clones encompassing this gene including its 5'- and 3'-flanking regions. Using a PCR-based genome walking strategy we obtained genomic DNA sequences that were analyzed for homology by dot matrix comparisons. Potential promoter and transcriptional regulatory motifs were identified using TESS and TRADAT software. We found that the 5'-untranslated region of *Gucy2d* is interrupted by one intron. This places the translation initiation site into exon 2 similar to the organization of guanylyl cyclase genes expressed in retinal photoreceptors. Further analysis of the upstream regions revealed putative transcription start sequences and olfactory-specific regulatory elements. These results will be useful in a systematic functional analysis of *Gucy2d* expression. Supported by NIH grant DC04281 to HJF.

αCAMKII EXPRESSION PATTERNS IN THE MOUSE MAIN OLFACTORY BULB

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CaMKII activity is important for the formation and function of synaptic connections. CaMKII is involved in LTP, spatial learning and memory, and neuronal plasticity in sensory systems, particularly visual and somatosensory cortex in the mouse. The roles of CaMKII in the olfactory system are less clear. CaMKII mRNA levels are high in the bulb, and very high in layer II of piriform cortex in the rat. However CaMKII expression patterns at the protein level have not been well documented. CaMKII activity has only been implicated in regulating odor adaptation in olfactory receptor neurons in the epithelium. In this study, we examined the normal expression patterns of αCaMKII in the mouse main olfactory bulb by immunocytochemistry. We found that CaMKII was selectively expressed in the GABAergic granule cells but not in the periglomerular cells, surprisingly CaMKII was not detected in the glutamatergic mitral/tufted cells. At the EM level, we further showed that CaMKII immunoreactivity is positive in granule cell spines and their dendrites, but negative in the opposing mitral/tufted cell dendrites in the reciprocal dendrodendritic synapses. In contrast, in the piriform cortex, like the majority of cortical regions, CaMKII was expressed in the glutamatergic neurons but not in the GABAergic neurons. Our results set the stage for our ongoing investigations on the roles of CaMKII activity in the formation and function of mammalian olfactory system.

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ULTRASTRUCTURAL LOCALIZATION OF THE GAP JUNCTION PROTEIN CONNEXIN43

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Communication via gap junctions is important to normal brain development, to glial cell functions, and in certain brain regions, to neural signaling. In the olfactory bulb, dye-coupling studies and immunohistochemical staining have provided evidence that gap junctions formed by the protein connexin43 (Cx43) might serve one or more of these functions. To identify the olfactory bulb cell types containing the Cx43 gap junctions, and therefore possible functions of the Cx43 junctions, salamander olfactory bulb sections were examined using pre-embedding immunoperoxidase methods and antisera against two different BSA-conjugated C-terminus peptides (amino acids 346-360, #18A from E. L. Hertzberg, Albert Einstein College of Medicine; amino acids 363–382, #C6219 from Sigma). Following dual pH 7.6 and 9.0 fixation, which produced punctate labeling of control rat olfactory bulb and heart sections, fine punctate labeling was distributed throughout the glomerular, external plexiform, mitral cell, and granule cell layers. Heavier but less dense punctate labeling occurred non-uniformly within the cellular layers and along their borders. At the ultrastructural level, the fine labeling within the cellular layers appeared to be localized to small glial cell processes, and the heavier labeling appeared to be localized to glial sheaths. The most prominent sheaths partially enveloped dendrites near synaptic sites and also neuronal cell bodies. These results indicate that the Cx43 gap junctions of the olfactory bulb are primarily localized to astroglial cells and therefore subserve non-neural functions.

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DIFFERENTIAL EXPRESSION OF G-PROTEINS IN THE OLFACTORY SYSTEM OF A REFLEX OVULATOR, THE PINE VOLE

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Odorant receptors play a role in guiding the formation of convergent axonal projections in the olfactory system. Since odorant receptors belong to the superfamily of heptahelical receptors, signaling via these receptors during axon sorting is expected to be mediated via regulatory G-proteins. Previously we have shown that segregation of axons expressing different G-protein alpha subunits occurs in the main and accessory olfactory bulb of the house mouse. Here we show that distribution of G proteins in the main olfactory system of the pine vole (microtus pinetorum), a reflex ovulator, is different from that of the mouse, a spontaneous ovulator. In the pine vole, olfactory neurons expressing Go project to glomeruli located in the apex of the olfactory bulb. This projections extend ventrally around the olfactory bulb and wraps around the lateral but never in the medial aspect of the bulb. Gi2 on the other hand is only expressed in the olfactory and vomeronasal nerve fiber. In the AOB, Gi2 is expressed prominently in the rostral region of the AOB, whereas expression of Go is confined to the caudal region confirming what has been shown in other rodent systems. Differential expression patterns of Go in the main olfactory projection suggest that these G-proteins may couple to odorant receptors and play a role in signal transduction during axon sorting and the formation of chemotopic glomerular connections.

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LOCALIZATION OF A LECTIN TO OLFACTORY NERVE ENSHEATHING AND SCHWANN CELL GLIA

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In the olfactory system plant lectins label subsets of olfactory receptor neuron (ORN) axons that are believed to express different carbohydrate epitopes. Moreover, an endogenous mammalian lectin, galectin-1, is found in the olfactory nerve on a specialized population of glia, ensheathing cells. The presence of both carbohydrate epitopes and a carbohydrate binding protein in the olfactory pathway raises the possibility that carbohydrate/protein interactions play a role in ORN axon fasciculation and/or guidance. One lectin, P. vulgaris isolectin E (PHA-E), labels ensheathing cells in mice, as demonstrated by its co-localization with p75 and laminin, common ensheathing cell markers. PHA-E labeling was also associated with the glial limitans of the olfactory bulb as well as connective tissue surrounding the bulb. At the electron microscope level PHA-E labeling was observed on ensheathing cell bodies and on small processes surrounding ORN axons. Cultured ensheathing cells also demonstrated strong PHA-E labeling, particularly in the Golgi apparatus, with weaker labeling throughout the cell body. Interestingly, PHA-E also labeled Schwann cells, peripheral glial cells morphologically similar to olfactory ensheathing cells. In the dorsal root ganglia, fine PHA-E labeled Schwann cell processes surrounded axons which were often grouped into larger bundles separated by thick bands of PHA-E labeling. The presence of PHA-E labeling in ensheathing and Schwann cells as well as in associated connective tissue suggests that the epitope to which PHA-E binds may be part of a general mechanism for restricting or guiding axons.

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DISTRIBUTION OF GO AND GOLF IMMUNOREACTIVITY IN THE OLFACTORY BULBS OF CATFISH

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In rodents, olfactory and vomeronasal receptor cells utilizing different G-proteins send axons to different territories of the main and accessory olfactory bulbs. In fish, three types of ORNs – ciliated and microvillar receptor cells and crypt cells – are intermingled within the single olfactory epithelium but project to different parts of the olfactory bulb (Morita and Finger, 1998, JCN 398:539-550). In the present study we used immunohistochemical methods to visualize G-protein subunits in order to test whether there is an obvious pattern of Go- and Golf-immunoreactivity in the olfactory bulb of catfishes. Paraformaldehyde-fixed cryostat sections and whole mount preparations were processed with polyclonal antibodies against Go and Golf and either fluorescent or biotinylated secondary antibodies. Our results show that Go-immunopositive ORNs project to two small sites in the olfactory bulb, one rostral-ventrally and one caudal-ventrally located. Strongly immunoreactive Golf axons project medially from the neuropil and disperse to many specific glomeruli localized medial and to a lesser extent lateral. Glomeruli are not immunoreactive for both Go and Golf processes. The relative amount of both axonal projections and immunoreactive glomeruli of Go versus Golf correspond to the proportion of immunoreactive ORNs of each type in the olfactory epithelium.Additionally DiI crystal placement in the respective Go and Golf immunopositive bulbar locations retrogradely label Go and Golf immunopositive ORN types. Supported by NIH Grant DC03792 (J. Caprio, P.I.).

GUSTATORY SENSITIVITY IN PATIENTS WITH ANOSMIA

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Patients with olfactory dysfunction often complain of decreased gustatory sensitivitiy. A total of 115 normosmic subjects / patients with olfactory/gustatory complaints participated in the study (44 f, 71 m; 17-81 years). Participants received testing with the "Sniffin' Sticks". For lateralized gustatory testing we used a novel procedure, developed by Kobal, Erlangen. Four concentrations each of NaCl, sucrose, citric acid, and caffeine are applied to the anterior portion of the extended tongue. Using a forced-choice procedure subjects indicate the perceived sensation. The sum of correct identifications was used as an indicator of gustatory sensitivity Gustatory scores differed ($p=0.001$) between normosmics (score=22.6), hyposmics (score=18.7), and anosmics (score=18.3). Small, but significant correlations between taste score and olfactory function were detected (discrimination: $r=.40$, identification: $r=.45$). The loss of gustatory sensitivity in patients with olfactory dysfunction may relate to central-nervous interactions between taste and smell.

EFFECT OF ORAL CAPSAICIN ON PERCEPTUAL RESPONSES TO TASTANTS

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A sensitive 2-AFC method coupled with bilateral intensity ratings tested the hypothesis that oral irritation modifies taste perception. Capsaicin (10 mM) was applied to one half of the tongue surface and KCl (100 mM) contralaterally. Subjects sipped 1 of 5 tastants and reported on which side of the tongue the taste was perceived as stronger. They also used a category scale to rate taste intensities. After a 15 min break, tastants were retested in the absence of irritation. In exp 1, tastants were (in mM) sucrose (300), citric acid (5.6), NaCl (300), quinine (0.1) and MSG (30). In exp 2, concentrations were lowered to 50, 0.1, 40, 0.01, and 10 mM, respectively. A significant majority of subjects chose the non-capsaicin treated side as stronger for both concentrations of sucrose (26/31 or 28/32; $p<0.001$) and quinine (22/31 or 26/32; $p<0.03$) and the low concentration of MSG (24/32; $p<0.01$). Mean intensity ratings were higher on the non-treated side for these sensations. No laterality differences were noted after the 15 min break. Thus, oral capsaicin reduces certain taste sensations but only when the irritant sensation is present. Whether this reflects a physiological action of capsaicin on taste processing, or a higher-level cognitive (e.g., masking) effect, remains to be determined.

ENHANCING ATHLETIC PERFORMANCE THROUGH THE ADMINISTRATION OF PEPPERMINT ODOR

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Previous research indicates odorant presentations can have both positive and negative effects on psychological perceptions of athletic task performance. The present study extends past research by assessing the effects of peppermint odor administration on actual athletic task performance. Forty athletes performed a series of physical tasks under conditions of no-odor or peppermint odor. The peppermint odor condition resulted in increases in running speed, hand grip strength, and number of push-ups, but had no effect on more skill-related tasks, such as basketball free-throw shots. 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 study was sponsored by a grant from NASA to the first author.

ODORS INFLUENCE SPEED OF TASTE NAMING

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The present experiment employs an analogue of the Stroop task to ask whether past flavor experience allows olfactory input to ready the cognitive system to expect a particular type of taste by testing whether naming a taste (sour) is faster in the presence of a matching smell (grapefruit) compared to one that does not match (cherry). Subjects were presented with an odor/taste pair and asked to say whether the taste was sweet or sour by quickly stopping a timer. Results were analyzed with measure of conflict (based on ratings of sourness, sweetness, and intensity) and block of presentation as independent variables in a GLM regression. Results indicated an effect of the interaction of conflict score and block on reaction time, with only performance on Block 1 showing the Stroop Effect. It appears that on initial presentation, subjects' perceptions of tastes were influenced by the presence of odors. This result suggests a sensory-free cognitive level at which flavors may be formed, but may also represent strategy changes based on higher task difficulty in block 1. During Blocks 2-6, it is hypothesized that subjects were either creating multiple new holistic flavor representations, each of which became an equally likely response, or processing the incoming flavor analytically, attending only to the taste portion of the flavor when it became advantageous to do so.

SENSORY FLAVOR-TEXTURE INTERACTIONS IN CUSTARD DESSERTS

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Possible effects of flavorants on specific oral texture sensations were explored for custard desserts. Four flavor sensations were selected from the sensory space of model custards, which could be largely described by two orthogonal dimensions running from meltiness to thickness and from roughness to creaminess, respectively. Vanilla sensations (represented in the second part by vanillin) were associated with creaminess and thickness, creamy sensations (diacetyl) with creaminess, bitter sensations (caffeine) with roughness, and almond sensations (benzaldehyde) with roughness and thickness. The causality of these associations was investigated in a seperate study, where the four flavorants were independently varied in model custards. The results indicated that only texture sensations that were part of the roughness/creaminess dimension were affected by some of these flavorants. Sensations that were part of this dimension, such as prickliness, and roughness were enhanced by added benzaldehyde, diacetyl, and caffeine, while others, such as fattiness and creaminess were suppressed by these flavorants. Sensations that were part of the other dimension, such as temperature, thickness, stickiness, and heterogeneity were virtually unaffected by these flavorants. The limitation of flavor effects to sensations that are part of the rough/creamy dimension may be coincidental or the common feature of the sensations that organizes them into the dimension may also make them "vulnerable" to the effects of flavorants. Alternative physiological and chemical explanations will also be given.

MISLOCALIZATION OF TASTES ACCOMPANIED BY TACTILE STIMULATION

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It was previously reported that taste can be mislocalized to the site of a moving tactile stimulus (Todrank & Bartoshuk, 1991). This effect was likened to "thermal referral", in which touch alters the localization of warmth and cold (Green, 1978). To investigate further how touch affects taste localization we adopted a paradigm similar to one used in the original studies of thermal referral. Solutions of 0.5M NaCl, 1.0M sucrose, 0.05M citric acid, 1.0mM quinine, and dH2O were delivered to the front edge of the tongue using three swabs spaced at 1-cm intervals. In a practice session subjects rated taste intensity (using the LMS) when only one of the three swabs contained a stimulus. In the test session tastes were delivered either (1) on the center swab only with dH2O on the outer swabs, or (2) on the outer two swabs with dH2O on the center swab. Subjects were told to rate taste intensity and quality at the center swab and to ignore any tastes at the outer swabs. The results showed that static tactile stimulation may disrupt taste localization in a manner analogous to thermal referral, i.e., perceived taste intensity at the center swab was not statistically different whether the swab contained dH2O or a taste. A tendency for "referral" to be strongest for quinine was not statistically significant. Experiments are continuing to determine, among other things, the spatial separation at which referral breaks down, and whether this distance differs as a function of taste quality.

ACQUISITION OF TEXTURAL AND TEMPERATURE AVERSIONS IN RATS WITH PARABRACHIAL NUCLEUS LESIONS

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It has been shown that rats with bilateral parabrachial nucleus (PBN) lesions fail to acquire an aversion to a gustatory conditioned stimulus (CS) when it is paired with a LiCl injection. The same rats can learn an aversion to oral trigeminal stimuli such as capsaicin or pure corn oil. Thus, the PBN appears to play an essential role in a standard conditioned taste aversion (CTA), but it may be less critical for learning aversions to other oral trigeminal stimuli. The present study was designed to determine whether rats with bilateral PBN lesions could acquire such an aversion. In a series of three different experiments, 28 rats (14 SHAM surgery, 14 PBNx) were given access to three different CS's having either salient textural or temperature cues (Purina wet mash, 10 °C water, pure corn oil). Half of the rats in each surgery group were then injected with either 0.15 M saline or 0.6 M LiCl. Daily post-conditioning tests were conducted where each animal received a two-choice test between the CS and and alternative stimulus. Results showed that while the LiCl-injected SHAM and PBNx rats acquired aversions to all of these substances, the aversions shown by PBNx rats were more transient than those acquired by the SHAM rats. Thus, these results clearly demonstrate that PBNx rats can associate different forms of trigeminal cues (and not gustatory cues) with LiCl-induced illness.

EFFECT OF ORAL STIMULATION ON POSTPRANDIAL THERMOGENSIS IN HUMANS

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Animal studies have demonstrated that oral sensory stimulation with food enhances postprandial thermogenesis. Inconsistent findings have been reported in humans. The present study determined whether an oral stimuli equal in palatability but varying in macronutrient composition differentially augments postprandial thermogenesis. Sixteen healthy adults (8 male, 8 female) participated in 6 test sessions conducted weekly each after an overnight fast. Sessions were administered randomly and included: ingestion of 50 grams of butter in capsules (to avoid oral fat stimulation) with 500ml of water within 15 minutes followed by oral stimulation with one of the following foods on a cracker: butter, highly unsaturated margarine, jelly, highly unsaturated margarine + jelly, cracker alone, or no oral stimulation. Sensory stimulation entailed masticating and expectorating 4.5g portions of each sample every 3 minutes for 110 minutes. Postprandial thermogenesis was assessed by indirect calorimetry intermittently at 35 and 85 minutes from the start of oral stimulation and continuously from 110 to 440 minutes. Postprandial thermogenesis was significantly greater than baseline for all treatments during the 440 minute period. While other treatment effects were observed, postprandial thermogenesis was similar after each oral stimulus during the 110 minutes of modified sham feeding and the entire 440 minute period. These data suggest that the macronutrient composition of an oral stimulus does not affect the cephalic phase postprandial thermogenesis.

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AGE-DEPENDENCY OF SUCROSE-ANALGESIA IN NEONATAL RATS

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Treatment of pain in newborns is associated with problematic drug side effects. Previous studies show that intraoral sucrose is effective in alleviating pain in human infants. However, the ability of intraoral sucrose to alleviate pain across the first 3 postnatal weeks is unknown. Here, we investigated effects of intraoral sucrose (7.5%) on withdrawal responses to thermal and mechanical stimuli in P0-21 rats. In some rats, Complete Freund's adjuvant (CFA) was injected in a forepaw or hindpaw to produce inflammation. For thermal stimuli, sucrose-induced analgesia (SIA) emerged at P3, peaked at P7-10, then progressively declined and was absent by P17/18. For mechanical forepaw stimuli, SIA emerged ~P10 and was absent at P17. By contrast, SIA for the hindpaw emerged at P13, although it was also absent at P17. In inflamed animals, sucrose reduced allodynia and hyperalgesia as assessed with mechanical stimuli. SIA in inflamed animals was present at P3 for the forepaw and P13 for the hindpaw, and was absent by P17 for both limbs. Taken together, these results indicate that intraoral sucrose alleviates acute pain in response to thermal and mechanical stimuli, and also effectively reduces inflammatory allodynia and hyperalgesia. SIA is age-dependent, emerging at ~P3-10 (for the forelimb) and is absent by weaning. The differential maturation of SIA for the fore- vs. hindpaw may be due to developmental changes in descending pain modulatory circuits. Support: PHS Grants DC03895, DE07309, DE11964.

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A REVIEW OF THE INFLUENCES OF CARDIOVASCULAR AGENTS ON TASTE AND SMELL PERCEPTION

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Altered taste and smell function can be very debilitating for patients, particularly the elderly. It is now apparent that many modern cardiovascular agents (according to the Physician's Desk Reference or PDR, > 25%) produce changes in chemosensory perception. Included are losses or distortions of taste qualities and the induction of phantom dysgeusias and dysosmias (e.g., the presence of a persistent sour, salty or bitter taste). Such sensory aberrations lead not only to diminished quality of life, but also to decreased food intake, loss of appetite, weight decrement, and depression. In this presentation, we summarize our review of this problem, including an assessment of prevalence, the physiological mechanisms likely involved, and the problems encountered by physicians and drug manufacturers in classifying and categorizing complaints of chemosensory side effects. This paper was supported, in part, by Grants PO1 DC 00161, RO1 DC 04278, RO1 DC 02974, and RO1 AG 27496 from the National Institutes of Health, Bethesda, MD USA.

367 Poster XLIX. Functional Characteristics of Sensory Cells

THE SEPTAL ORGAN, AN ATTRACTIVE MODEL SYSTEM FOR OLFACTORY CODING STUDY

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Functional studies of olfactory coding in mammals have been hindered by the inherent complexity of the system; there are millions of receptor neurons each of which expresses only 1/1000 odorant receptors and thousands of odorants. Assessing the relationship between odorants and subpopulations of receptor neurons may be facilitated with the establishment of a simpler model system. The septal organ (organ of Masera) is a small island of olfactory epithelium surrounded by respiratory epithelium, lying near the base of the nasal septum at the entrance to the nasopharynx. The septal organ and the main olfactory epithelium (MOE) resemble each other in their cellular compositions, in their responses to odorants, and in their projection to the main olfactory bulb as shown by our DiI tracing experiments. We have further demonstrated that the cAMP pathway is the key signal transduction mechanism in the septal organ as in the MOE. However, relative to the MOE, the septal organ is a much simpler system with only about 20,000 neurons expressing fewer than 10 receptor genes in an adult mouse. Physiological recordings are greatly facilitated by the fact that there are only a few subtypes of receptor neurons in such a restricted area (~1% of the MOE). We have begun to classify the receptor neurons in this area according to their odor response spectra. Functional and molecular characterization of the septal organ is providing critical information underlying olfactory coding strategies which may also shed light on the still unknown behavioral significance of this organ.

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RESPONSES OF SINGLE CHORDA TYMPANI TASTE FIBERS OF THE CALF

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Palatability has a major influence on feed intake in ruminants and it becomes more important in current conditions of confinement housing and management. Many feed additives were formulated based on human taste perception. However, cattle taste system is not well studied. We recorded activity of 56 chorda tympani single fibers in calf during tongue stimulation with 3 salts, umami compounds, 4 acids, 4 bitter compounds and 17 sweeteners. Hierarchical cluster analysis revealed 4 different clusters. N and H clusters were most populous (38% and 39% accordingly) and formed by NaCl- and acid-best fibers. Amiloride suppressed responses to NaCl in 12 out of 14 tested N fibers and only 3 out of 9 H fibers. Only 7% of fibers were responsive to sweeteners and 16% of fibers were most sensitive to bitter compounds. Comparison of response profiles of each cluster demonstrated that the calf taste world is quite different and conclusions based on human perception cannot be applied to this animals. For example, aspartame, which is sweet to humans, stimulated H and did not stimulate S fibers. All acids tested (including propionic and butyric, which are the products of fermentation in rumen) were the most powerful stimuli for calf CT fibers. Very high concentrations of denatonium benzoate were needed (5 or 10mM) to stimulate Q fibers.

ELECTRICAL PROPERTIES OF FOUR TYPES OF CELLS IN THE BULLFROG FUNGIFORM PAPILLAE

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We made a direct correlation between the morphological and physiological features of cells composing the taste epithelium. Whole-cell patch clamp method was applied to cells in the slice preparation made from the bullfrog papilla. Four types of cells were morphologically identified by Lucifer yellow staining. Type Ia (mucous) cells showed spherical shape. They had linear I-V relations, and were dye-coupled with each other. 10 mM quinine did not cause any detectable response in type Ia cells. Type Ib (wing) cells were characterized from 3 fin-like apical processes. They showed voltage-gated Na+ and K+ currents under the voltage clamp, and generated action potentials under the current clamp condition. They responded to quinine with inward current when the membrane was clamped at -60 mV. Type II cells were characterized by a thick (2-3 μm) dendrite running to the surface. They had voltage-gated Na+ and K+ currents, and generated action potentials. They responded to quinine. Type III cells had thin dendrite of sub-micrometer diameter. They had voltage-gated Na+ and K+ currents and generated action potentials, but not responded to quinine. Type Ib and type II cells had larger Na+ current than type III cells, indicating that type III cells may be unique in terms of electrical excitabilities. This slice preparation could be a powerful tool to further investigate the taste system.

IS ROMK1 ($K_{IR}1.1$) THE ELUSIVE APICAL K^+ CHANNEL IN MAMMALIAN TASTE CELLS?

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Apical K^+ channels in taste receptor cells have been demonstrated in lower vertebrates to play a role in acid and bitter taste transduction. It has been hypothesized that apical K^+ channels may serve a similar function in mammalian taste cells, yet there has been no direct evidence in support of an apical K^+ conductance. Using probes for various types of K^+ channels, we have identified the presence of a weak inwardly rectifying K^+ channel known as ROMK1 ($K_{ir}1.1$) in RNA from taste buds of the fungiform, foliate and vallate papillae in rat. ROMK channels are involved in maintenance of K^+ homeostasis, K^+ excretion and maintenance of the resting potential and, as such, are intriguing candidates for modulation by tastants. We have sequenced the PCR products (~447 bp) and they show a high degree of homology (>95%) between the taste ROMK1 channel and that found in kidney. To determine its localization within the taste bud, we have used commercially available antibodies against ROMK1 to label isolated taste buds from the three papillae in rat tongue. Using confocal microscopy, ROMK1 labeling was clearly evident on the apical membranes of the taste cells, particularly those from the posterior tongue. Additionally, diffuse labeling was seen on the basolateral membranes suggesting that this channel may not be entirely apically restricted. Presently, we are using patch clamp recording on rat taste cells to characterize this inwardly rectifying conductance and its modulation by tastants. *Support was provided by NIH DK55809 (TAG).*

LONG TERM RECORDINGS FROM SINGLE CHORDA TYMPANI NERVE FIBERS IN THE RAT

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Recently we have reported on long-term recordings from the whole chorda tympani nerve (CT) using a cuff electrode (Soc. Neurosci. Abstr, 26:2000). In these recordings the cuff electrode was implanted close to the point at which the CT branches from the lingual nerve. In preliminary experiments from these implants in awake behaving animals, the recordings were contaminated by EMG signals from the oral-facial muscles. This problem was solved by moving the recording site to the location where the CT crosses the middle ear. In these experiments a two wire Pt-Ir electrode was implanted around the CT in the middle ear and connected to a skull-mounted plug. To record from a limited number of fibers the CT was first dissected into a thin bundle. After ~ 2 weeks implantation the animal was briefly reanesthetized, connected to a recording apparatus, and mechanical and standard taste stimuli flowed over the anterior tongue. Recordings were repeated daily. Although the recordings from these small bundles were usually multi-unit, it was possible to identify single units by amplitude and waveform. The single units responded to either mechanical or chemical stimuli and some responded to both. Recording from the same single unit could be made for over one week. For some of the units the response pattern to chemical stimulation varied from day to day, while in other units the response characteristics were more constant. The variability in the response of these units may relate to the "aging" of the taste cells during taste bud turnover. Supported by a grant DC04198 from NIDCD.

ELECTROPHYSIOLOGICAL AND CHEMOSENSORY PROPERTIES OF CULTURED TRIGEMINAL NEURONS

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Trigeminal nerve fibers innervating the facial mucous membranes are sensitive to chemical stimuli. Additionally to the indication of protective reflexes the trigeminal system can mediate odour sensations as shown psychophysically in humans with damaged olfactory system. How trigeminal neurons acquire and mediate smell information is still unknown. We established a culture system of the rat Ganglion gasseri to investigate the trigeminal chemoperception electrophysiologically at single cell level. Cultured neurons were identified morphologically, electrophysiologically and by positive immunostaining with an antibody recognizing the neuron specific tubulin. The estimated ratio of non-neuronal to neuronal cells was about 100 to 1. The trigeminal neurons showed spherical somata of about 45 μm in diameter and a remarkable neurite outgrowth within one day after dissociation. The patch-clamp technique was used to investigate the biophysical properties of the cultured trigeminal neurons: The resting membrane potential averaged ?51 mV. No spontaneous activity could be detected but single action potentials could be generated by current injection. Hyperpolarization to potentials more negative than ?65 mV produced a clear sag in 69% of the cells indicating the presence of Ih channels. In further experiments we intend to investigate the effect of various odors and known trigeminal stimuli on individual cultured trigeminal neurons. The sensitivity and specificity of the cells as well as the involved signal transduction mechanisms will be studied.

EXPOSURE TO ODORS OR THE PDE4 INHIBITOR ROLIPRAM INDUCES PHOSPHORYLATION OF CREB IN THE MOUSE OLFACTORY BULB

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In the brain, increases in cAMP can result in phosphorylation of the cAMP-responsive binding protein (pCREB), leading to consequent changes in gene expression and synaptic plasticity. However, in the olfactory system, long term effects of cAMP induction following exposure to odors are largely unknown. We examined whether pCREB could be induced in the mouse olfactory system after either exposure to odors or pharmacological elevation of cAMP by administration of the PDE4 inhibitor rolipram. Male mice were injected with 0.5 mg/kg rolipram or saline 30 min before being exposed to 2% ethanol (control) or a mixture of hexanol, eugenol and citronellal in 2% ethanol for 20 min. Olfactory tissues were then quickly isolated and processed for Western blotting using anti-pCREB antibodies (NEB). Consistent with previous results in the rat (Moon et al., PNAS 96:14605, 1999), olfactory epithelium (OE) in saline-treated mice showed elevated pCREB levels in response to odorants ($p<0.005$); however, rolipram had no effect on pCREB levels in the OE of odor- or ethanol-exposed subjects. By contrast, in the olfactory bulb (OB), pCREB levels were increased following either odor exposure ($p<0.005$) or rolipram administration ($p<0.03$) relative to controls. However, rolipram treatment did not further increase the levels of pCREB in the OB seen in response to odors. These results indicate that elevation of cAMP after exposure to odors induces pCREB in both the OE and OB of mice. Rolipram treatment alone mimicked the effect of odors on pCREB levels in the OB, but not the OE. Supported by DC03019.

INHIBITORY ODOR RESPONSES RECORDED EXTRACELLULARLY FROM TOAD OLFACTORY EPITHELIUM

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We have previously reported that dissociated toad (C. Caudiverbera) olfactory receptor neurons exhibit odorant-induced excitatory and inhibitory responses, expressed as increases and decreases of their discharge rates (1). We investigated whether similar responses can be observed in the intact olfactory epithelium. Extracellular recordings using low impedance tungsten electrodes allowed us to resolve the activity of 1-3 neurons at a time. We developed a software that allowed us to separate the activity of each of the neurons contributing to every particular recording, and independently examine the behavior of every cell. We found 37 of 40 neurons that responded to single odorants (geraniol, isoamyl acetate or citronellal) gave excitatory responses, while the other 3 were inhibited by the odorant (8%). Our results are consistent with the notion that inhibitory odor responses are part of the normal behavior of olfactory receptor neurons, in addition to excitatory responses. (1) Morales, B, Ugarte, G, Labarca, P, Bacigalupo, J. 1994. Proc. R. Soc. London B 257: 235-242. Supported by Fondecyt 1990938, Presidential Chair in Science and grant ICM P99-031-F (JB).

THE USE OF FRESH CADAVERIC HUMAN OLFACTORY EPITHELIUM FOR PHYSIOLOGIC STUDY

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The study of human olfaction at the level of the receptor cells has been restricted by the relative difficulty in obtaining the tissue from live patients and the small amount of tissue that is obtained upon biopsy. As an alternative, a fresh cadaveric isolation system has been developed to study olfactory epithelium/receptor physiology. The olfactory receptor cells (ORCs) obtained from this system are comparable to the anatomy and physiology that is known of ORCs from live patients. ORCs can be isolated with the same morphologic characteristics as *in vivo* ORCs, demonstrating intact cell bodies with dendritic extensions and dendritic bulbs that have identifiable olfactory cilia attached. Using intact olfactory epithelium and EOG recording techniques, odorant responses can be obtained. The features of these responses are similar to those obtained from live patients (Leopold et al., Laryngoscope 110: 417-421, 2000). Dose-response curves to various odorants can be elicited and adaptation also is seen. Furthermore, isolated ORCs can exhibit the expected membrane properties of excitable cells and reveal inward and outward membrane currents by perforated patch recording techniques. Some of these isolated cells can produce responses to odorants. Overall, the fresh cadaveric isolation system is likely to prove to be a good model system for physiologic study of the olfactory epithelium.

KERATIN 18 EXPRESSION IN LIGHT CELLS OF RAT TASTE BUDS

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At the electron microscopic level, two distinct taste bud cell types have been identified that differ markedly in their morphologies (Pumplin et al., *J Comp Neurol* 378: 389-410, 1997). Dark (Type I) cells project thin cytoplasmic lamellae that wrap around neighboring cells, whereas light (Type II) cells are characteristically round in transverse section. These distinct shapes can be seen at the light microscopic level using immunohistochemical markers when the taste buds are sectioned transversely to their long axis. For example, gustducin and NCAM are expressed only on separate subsets of round (light) cells. Keratin 18 (K18) is present in a subset of taste cells, which comprise about 25% of the cells and which are among the oldest cells in the vallate taste buds (Zhang et al., *Differentiation* 59: 155-162, 1995). These cells appear to be light cells on the basis of their shapes. Morphometric analysis shows that the shape factor distribution of K18+ cells of transversely sectioned rat vallate taste buds overlapped that of light cells but not dark cells previously measured at the EM level. To further confirm the characterization of K18+ cells, we conducted double-labeling experiments. All gustducin+ taste cells were also K18+, although there were many K18+ cells that did not express gustducin. Further studies are underway with additional antibodies to determine whether K18 may be a marker for all light cells. Supported by NIDCD DC00347 to D.V.S.

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IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES OF PGP 9.5 IN THE TASTE BUDS OF RAT CIRCUMVALLATE PAPILLLAE

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Protein gene product 9.5 (PGP 9.5) is a neuron-specific protein that is used as a marker for some neurons and neuroendocrine cells. Its functional significance is still unclear. Olfactory receptor cells display immunoreactivity (IR) to PGP 9.5, but little is known about PGP 9.5 in taste buds. Our studies show that PGP 9.5-IR is present in many taste cells and intragemmal nerve processes. Cells immunoreactive to PGP 9.5 do not colocalize with gustducin-IR cells, but a few do colocalize with a subset of serotonin-IR taste cells. Results from immunoelectron microscopy indicate that two types of taste cells display PGP 9.5-IR. One cell type possesses a circular nuclear profile and terminates in a group of small microvilli, characteristic of type II cells. Another type of PGP 9.5-IR taste cell has a slender, invaginated nucleus, extends one thick microvillus into the taste pore, and possesses synaptic vesicles. Based on these results, we tentatively classify this cell as a "rat type III taste cell". All of the synapses we observed (75) were associated with the rat type III taste cells. Quantitative results indicate that the nuclear shape factor ($p<0.001$) and ellipticity ($p<0.001$) are significantly different between the nuclei of two types of cells. These findings demonstrate that more than one cell type displays PGP 9.5-IR. Supported in part by NIH grants DC00285 and DC00244.

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BONE MORPHOGENIC PROTEIN 4 (BMP-4) IS PRESENT IN TASTE CELLS OF ADULT MICE

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BMP-4 is present in taste bud precursors at E12 and is associated with lingual papillary formation (Jung et al., Mech dev 81: 179-182 1999). At early prenatal stages (P1), BMP-4 is restricted to 3-4 elongate taste cells (Hall, J.M. et al., Chem. Senses 25: 627, 2000). BMP-4 continues to be expressed in some taste cells of adult mice. The function of BMP-4 in adult taste cells is unknown. Whether BMP-4 is present only in newly divided immature taste cells, or whether it is present in phenotypically mature type I or type II cells is unknown. Different taste cell types have been identified based on morphological and immunohistochemical criteria. Morphologically taste cells can be divided into Type I and Type II cells; histochemically many different taste cell types exist. Immunohistochemical markers such as blood group H antigen and gustducin are present in type I and type II cells respectively. To determine which taste cell types express BMP-4 and whether mature type I or type II taste cells express BMP-4, we have used immunohistochemical markers including ubiquitin-carboxyl terminal hydrolase, neural cell adhesion molecule, gustducin, serotonin, SNAP-25 and anti-blood group H to identify taste cell types gene targeted in BMP-4^{lacZ} "knock-in" mice. In these mice, the BMP-4 promoter drives Beta-galactosidase (β-gal) expression. β-gal colocalizes with PGP 9.5 and N-CAM but is absent from gustducin immunoreactive (IR) taste cells. Morphologically, β-gal is located in long, slender taste cells. BMP-4 is present in differentiated type II non-gustducin-IR taste cells.

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IMMUNOCYTOCHEMICAL ANALYSIS OF TASTE CELLS INFECTED WITH VIRAL VECTORS

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Previous studies in our lab demonstrated that EGFP is expressed in a subset of cells following infection of isolated rat taste buds with an adenovirus vector (Ad5-CMV-EGFP). The current study was undertaken to examine more closely the distribution of EGFP in infected taste buds. In addition, EGFP expression following infection with a herpes (HSV-1) vector was compared to EGFP expression following infection with the adenovirus vector. To do these studies, circumvallate and foliate taste buds were isolated from 6-8 week old male Sprague Dawley rats. Immediately following isolation, taste buds were infected with either the adenovirus vector or the herpes (HSV-1) vector and incubated at 35 ° C for 17-24 hours. The infected taste buds were then fixed in 4% paraformaldehyde and subsequently processed for immunocytochemistry. Antibodies against several proteins were used and the subpopulations of cells expressing EGFP and each antigen were compared. Our studies indicate that both adenovirus and herpes vectors produce EGFP in infected taste buds. However, infection with the HSV-1 vector resulted in EGFP expression in a more diverse population of taste cells, relative to infection with the adenovirus vector.

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GUSTDUCIN IMMUNOREACTIVE TASTE CELLS IN MOUSE CIRCUMVALLATE PAPILLAE DO NOT EXPRESS SNAP-25

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Gustducin is a transducin-like G protein expressed in rodent taste receptor cells, as well as in chemosensory cells in the gut and vomernasal organ. α-Gustducin knockout mice exhibit reduced behavioral and nerve responses to bitter and some sweet compounds. Recently we documented the presence of gustducin in microvilli of Type II taste cells (Yang et al., 2000). In that study, we did not observe any synapses between gustducin-immunoreactive (IR) taste cells and nerve fibers, although a few synaptic vesicles were seen in the gustducin-IR cells. We hypothesize that classical synapses are not present between gustducin-IR taste cells and nerve fibers. Thus, presynaptic membrane proteins like SNAP-25 will probably not be expressed in gustducin-IR taste cells. Because we observe some synaptic vesicles in gustducin-IR cells, we speculate that the synaptic vesicle proteins, synaptobrevin and synaptotagmin, will be present in gustducin-IR taste cells. Our immunocytochemical results show that SNAP-25-IR does not colocalize with gustducin-IR in taste buds, but synaptobrevin-IR and synaptotagmin-IR do colocalize with gustducin-IR, as predicted. We are currently doing in situ hybridization with SNAP-25b RNA probe followed by an immunocytochemical colocalization step to confirm our immunocytochemical results. If gustducin-IR cells lack classical synapses as hypothesized, we believe they may possess non-classical sites of synaptic vesicle release. Supported in part by NIH grants DC00285 and DC00244.

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CLONING OF FUCOSYLTRANSFERASES RELATED TO LEWIS^B FROM A RAT TASTE ENRICHED CDNA LIBRARY

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Fully operational taste receptor cells should express both ion channels or second-messenger systems for excitation in response to tastants and presynaptic proteins involved in neurotransmitter release for communication with afferent nerves. In rat taste buds, the carbohydrate epitope Lewis^b appears only on the surfaces of light cells that express gustducin (a G-protein involved in responses to sweet and bitter substances), but do not express presynaptic proteins, suggesting an intermediate developmental stage. Lewis^b is absent from light cells expressing presynaptic proteins. Labeling of dividing cells with bromodeoxyuridine shows that expression of Lewis^b in vallate taste-bud cells begins between 6.5 and 8.5 days after the last cell division, 3-4 days after the onset of gustducin expression. Thus, expression of fucosyltransferases responsible for synthesizing the Lewis^b epitope is both cell-type specific and developmentally regulated. Synthesis of Lewis^b in humans requires addition of two fucoses, catalyzed respectively by fucosyltransferases encoded by the FUT2 and FUT3 genes. I obtained clones of FTB, the rat homolog of FUT2, from the rat taste-bud cDNA library prepared by Dr. N. Ryba. The coding region and 3'-untranslated region of these clones appeared essentially identical to that of FTB cloned from a rat colon cancer cell line (GenBank AB006138). However, one clone contained an insert of 107 base pairs in the 5' untranslated region that appears to be an additional exon not present in mRNA for colon-cell FTB. Supported by the Bressler Foundation, Univ. of Maryland, Baltimore.

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CHARACTERISTICS OF FUNGIFORM TASTE BUDS AND CELLS 7-17 DAYS FOLLOWING EXPOSURE TO β-RADIATION

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The phenomenon of taste loss is a frequent but little addressed complaint of people at many stages of life, growth, aging, and illness. However, personal accounts of taste loss from individuals can be devastating. The development of the tongue-irradiator facilitates investigation of various aspects of radiation on lingual epithelium and taste cells after exposure to β-radiation. *Sprague dawley* rats received 6, 12, or 18 Gy of radiation to the dorsal tongue. Tissue was examined at 7, 10, and 17 days following the exposure. Histologically, the surface epithelium thickened, died, and sloughed off over the 17 day course, with severity related to dose. Taste bud numbers and volume decreased until day 10 where they leveled off. Within the fungiform taste buds, the cells take on an epitheloid apperance. At day 17 most of the buds are small and atrophic, and are contained within the sloughing epithelium. Interestingly, new taste buds can be seen arising in the regenerating epithelium. Utilizing immunohistochemistry, irradiated taste cells contain one Bax-positive cell, 6-8 gustducin-positive cells, and an increase in the number of NCAM-positive cells over normal. These data suggest complex mechanisms of taste cell alteration after radiation which may indicate mechanisms of taste loss.

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AMINO ACID IMMUNOREACTIVITY OF CHANNEL CATFISH PERIPHERAL TASTE SYSTEM

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Immunocytochemical techniques were used to investigate the amino acid profiles of channel catfish taste buds innervated by facial (maxillary barbel) and vagus (2nd gill arch) nerves. Taste epithelia was gluteraldehyde fixed, embedded in plastic, and serially sectioned (50-100 nm). Primary antibodies against alanine (Ala), L-aspartate (Asp), GABA, glutamate (Glu), glutathione (GSH), and taurine (Tau) were applied to deplastized sections. Immunoreactivity (IR) was visualized following application of nanogold secondary antibody and silver intensification. Amino acid IR revealed a diversity of cell types. Most Glu IR was observed in nerve layer, the peduncle and base of the taste bud. A few cells in the taste buds were Glu positive (Glu+). Glu IR was similar in barbel and gill arch taste buds. Asp IR was also noted in the base of the taste bud but only partially co-localized with Glu IR. GABA IR was observed in receptor cells, in the apex of the peduncle and the nerve layer, and co-localized with Glu IR at the apex of the peduncle. More GABA IR receptor cells were noted in vagal innervated taste buds. GABA and Glu IR colocalized in the apex of the peduncle. Tau IR was greatest in lateral receptor cells. GSH IR was restricted to a few receptor cells in the central taste bud and to a halo of GSH IR surrounding taste buds. Both elongated and round cells were Ala IR, some were GABA+. Vagal innervated taste buds displayed stronger Ala IR. Our data suggests that cells with a wide diversity of metabolic phenotypes are found in taste buds. Funded by NIH grants DC01418 and NS-07938.

384 Poster LI. Second Messengers

CHEMORESPONSE IN PARAMECIUM: INVOLVEMENT OF THE CALCIUM PUMP

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Paramecia are attracted to stimuli through multiple transduction pathways; two involve the plasma membrane calcium pump (PMCA) to sustain hyperpolarization. We used antisense technology to down regulate levels of calmodulin, an activator of the pump and found inhibition the chemoresponse to glutamate and variably to acetate (Chem. Senses 21: 55-58, 1996; Chem. Senses 24: 1998). There are 3 PMCA genes in Paramecium; here we discuss isoform 2. The C terminal calmodulin binding domains (CBDs) of the calcium pumps have potential PKA and PKC sites, and phosphorylation or calmodulin binding to the CBD activates the pump. We mutanted the CBD, with alanines or glutamates substituted for the serines of the putative PKA phosphorylation site. Wild type CBD binds calmodulin and is phosphorylated by PKA and PKC in vitro. Mutant CBDs are not phosphorylated, implying that the two serines that we mutated are the sole PKC and PKA phosphorylation sites for the CBDs. Mutant CBD with alanine substitutions binds to calmodulin, but the mutant CBD with glutamates does not. In vitro assays of the pump ATPase activity show that the pump is activated by calmodulin and kinases; all 3 expressed CBDs, wild type and mutants, inhibit ATPase activity in vitro. We are exploring these unexpected results to help interpret the over-expression of CBDs in Paramecium cells. In preliminary results, over-expression of wild type CBD inhibits chemoresponse to glutamate, folate and to a variable extent, to acetate. Supported by NIH DC00721 and VCC.

CHEMOSIGNAL TRANSDUCTION IN THE VOMERONASAL ORGAN OF GARTER SNAKES: CHEMOATTRACTANT-INDUCED IP3-MEDIATED CALCIUM RELEASE

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In snakes, the response to non-volatile prey chemicals is mediated by the vomeronasal system and this has permitted us to isolate and purify specific prey extracts to be used as selective chemoattractant stimuli. Using optical techniques and fluorescent Ca2+ indicators, we here describe a novel mechanism mediating chemosensory transduction in snake VN receptor neurons. We found that chemoattractants produce a transient IP3-related cytosolic accumulation of [Ca2+]i in the dendritic tips and somata of the neurons via two pathways: Ca2+ release from IP3-sensitive intracellular stores and, to a lesser extent, Ca2+ influx through the plasma membrane. The initial chemoatractant cytosolic Ca2+ elevation can be demonstrated even in the absence of extracellular Ca2+. Once responses have been triggered, other mechanisms also contribute to the generation and maintenance of these cytosolic Ca2+ transients. In regions close to the cell bodies of the receptor cells, the initial elevation of cytosolic Ca2+ is further boosted by the activation of voltage-sensitive Ca2+ channels (VSCC) and a Ca2+-induced Ca2+ release (CICR) from intracellular ryanodine-sensitive stores.Return of Ca2+ to prestimulation levels may involve Ca2+ extrusion mediated by a Na+/Ca2+ exchanger. Supported by Grants #DC02531,NIH to MH & #9905700,NSF to DW.

PHYSIOLOGICAL, AND PHARMACOLOGICAL EVIDENCE FOR ODOR STIMULATED PHOSPHATYDILINOSITOL 3-KINASE IN LOBSTER OLFACTORY RECEPTOR NEURONS

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Two antagonists of phosphoinositide 3-OH kinases (PI3Ks), LY294002 and Wortmannin, reduced the magnitude of the receptor potential in lobster olfactory receptor neurons (ORNs) recorded by whole-cell patch-clamping the cells in situ. A molecule of the predicted size can be localized by Western blotting and immunocytochemical staining to the transduction zone with an antibody raised against the c-terminus of human PI3K-P110β. Two D-3 phosphorylated inositol lipids, PI(3,4)P2 (1-4 mM) and PI(3,4,5)P3 (1-4 μM) applied to the cytoplasmic side of inside-out patches taken from cultured lobster ORNs reversibly activated a Na^+-gated channel previously implicated in the transduction cascade in these cells. D-3 phosphorylated inositol lipids were the most effective phosphoinositides (1 mM) in enhancing the open probability of the channel. Collectively, these results implicate one or more D-3 phosphorylated inositol lipids in lobster olfactory transduction, and raise the need to consider PI3K-dependent PI signaling as a functional mechanism in olfactory transduction.

IMMUNOHISTOCHEMICAL IDENTIFICATION OF COMPONENTS OF THE CHEMOATTRACTANT SIGNAL TRANSDUCTION PATHWAY IN VOMERONASAL BIPOLAR NEURONS OF GARTER SNAKES

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The chemosignal transduction pathway in the vomeronasal epithelium of garter nsakes involves the generation of second messengers leading to transduction of the chemical signal to an electrical signal. The binding of chemoattractant and its receptors activates Gi/o-proteins which in turn activate PLC which cleaves PIP2 to DAG and IP3, resulting in a transient cytosolic Ca2+ increase. Data from calium imaging experiments suggest that the PI turn-over pathway plays a major role in this Ca2+ increase. It is known that IP3 activates this increase through IP3 receptors (IP3Rs). However, the chemoattractant-induced Ca2+ increase involves intracellular Ca2+ release as well as influx from extracellular Ca2+. It also suggests that the Ca2+ increase involves the activation of both IP3-sensitive and -insensitive receptors, i.e., IP3Rs and ryanodine receptors (RyRs) and there appears to be a differential distribution of these stores. We now provide evidence that the IP3Rs are largely distributed in the dendritic regions of the VN sensosry epithelium whereas the RyRs are confined to the somata region and practically absent in the dendritic region. In addition, a transient receptor protein (TRP) is concentrated in the dendritic region. Supported by NIH Grant No. DC20531 and NSF Grant No. IBN9905700.

SIGNAL TRANSDUCTION PATHWAYS IN ODORA CELLS

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Odora, an olfactory neuronal cell line, responds to an odorant mixture when transfected with odorant receptor "U131" (Murrell and Hunter, 1999). Previously we showed that Odora cells share some signaling components with olfactory sensory neurons. Here we investigate the pathways involved in the odorant response of U131-Odora cells. By Ca imaging, we demonstrated that the increase of intracellular Ca induced by odorant depends on extracellular Ca, but not on extracellular Na. The response to odorants was reversibly inhibited by phospholipase C (PLC) inhibitors: U73122 and neomycin. An IP3 receptor antagonist, Xestospongin C, also effectively blocked the odorant response reversibly. Odora cells also respond robustly to ATP; this response is not dependent on extracellular Ca, but releases Ca from intracellular stores. U73122 also blocked the ATP response, while the less active U73343 did not. ATP probably acts through a G protein-coupled P2y receptor. Furthermore, Odora cells did not respond to IBMX and forskolin. However, Odora cells transfected with adenylyl cyclase III showed elevated Ca in response to IBMX and forskolin. Stimulation of U131-Odora cells with odorants or ATP elevated IP3. Further, Western blot analysis demonstrated that IP3 receptor type I is present in the cells as well as in rat olfactory epithelium. Together, these data suggest that odorant responses of Odora cells are mediated by PLC signaling, an IP3R, and an as yet identified Ca channel(?) on the plasma membrane. We speculate that odorant receptors in vivo may couple to a similar transduction pathway to modulate odorant signaling or axon targeting to glomeruli in the bulb.

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TRP-T, A NOVEL TRP RELATED PROTEIN EXPRESSED IN TASTE RECEPTOR CELLS

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To transduce responses to gustatory stimuli taste receptor cells (TRCs) use a diversity of mechanisms including ion channels (for sodium salts and acids) and G-protein coupled receptor (GPCR) cascades (sugars/sweeteners, bitter alkaloid, glutamate-umami). Gustducin is a heterotrimeric G-protein selectively expressed in ~30% of TRCs; gustducin's α-subunit is crucial for the transduction of responses to sweet and bitter stimuli. We have gained further insights into the composition of the transduction pathways present in gustducin-positive TRCs by identifying genes preferentially expressed in gustducin-positive vs. gustducin-negative TRCs. Differential hybridization screening of a gustducin-positive TRC cDNA library, led us to the discovery of the novel G-protein γ subunit, γ13, that is co-expressed with α-gustducin. Here we describe the cloning and functional characterization of Trp-T, a novel member of the Trp (transient receptor potential) family of ion channels that co-expresses with α-gustducin and γ13. Functional analysis demonstrates the ability of Trp-T to function as an ion channel; pharmacological properties of Trp-T and its putative role as a downstream effector in TRC physiology are discussed. Supported by: NIH DC03055 and DC03155 (R.F.M.); MH57241 (M.M.); and DC00310 (L.H.). RFM is an Investigator of the Howard Hughes Medical Institute.

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SIGNALING COMPONENTS OF THE IP_3 PATHWAY IN RODENT TASTE CELLS

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Taste receptor cells use a variety of different second messengers to transduce chemical stimuli into neural signals. One of these second messengers is inositol trisphosphate (IP_3), believed to be involved in both bitter and sweet taste transduction. We previously reported immunocytochemical evidence that IP_3R-3 is the prevalent IP_3R isoform in taste cells. Furthermore, all α-gustducin immunoreactive taste cells that we examined also exhibited IP_3R-3 immunoreactivity. In contrast, a subset of taste cells expressed IP_3R-3 immunoreactivity but lacked α-gustducin immunoreactivity. The current study is a continuation of the immunohistochemical characterization of taste cells expressing IP_3R-3 immunoreactivity. We used double label immunocytochemistry to show that γ13 and IP_3R-3 are found in the same subset of taste cells. In addition, IP_3R-3 and PLCβ2 antibodies label the same subset of taste cells. Recent evidence suggests that a transient receptor protein (Trp) channel, Trp-T, is located on the plasma membrane of taste cells and may mediate Ca^{2+} influx in response to bitter stimuli (Perez et al., AChemS 2001; Ogura and Kinnamon, AChemS 2001). We show that Trp-T immunoreactivity labels the same subset of cells as IP_3R-3, indicating that Trp-T is an integral part of the IP_3 signaling pathway. Supported by NIH grants DC7066 and DC00244.

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MIXTURE SUPPRESSION AND ODOR SUPPRESSION OF CAMP-INDUCED CURRENT IN OLFACTORY RECEPTOR NEURONS

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Many studies in vertebrate olfactory receptor neurons (ORNs) show that mutual suppression between odorants can be observed when multiple odorants are applied simultaneously. However, underlying mechanisms of this mutual suppression are not clear, and it is unknown how mutual suppression plays a role in the olfactory system. Here we investigated the nature of suppression of the transduction current induced by odorants and further examined the possibility that the odor inhibitory effects have a role in the mutual suppression in newt (*Cynops pyrrhogaster*) ORNs. Using whole-cell patch-clamp method, we recorded that odor stimulation could suppress IBMX-, cAMP-, and 8-Br-cGMP-induced current. These results show that odorants could directly suppress CNG-channels, and further comparison of the ratio of current suppression suggested that odor suppression of the transduction current is mainly due to direct suppression of CNG-channels. The odor inhibitory effect was induced by various odorants (anisole, isoamyl acetate, cineole, limonene, and isovaleric acid) in dose-dependent manner. Furthermore, we observed the depolarization caused by isoamyl acetate was inhibited by anisole in cells that were excited by isoamyl acetate, indicating that one odorant could suppress an excitatory response induced by another odorant. To ensure the mutual suppression of responses of ORNs in olfactory epithelium, we recorded odorant-evoked voltage changes (EOG) across the olfactory epithelium of the newt. The results of EOG recording are consistent with the result obtained from individual neurons.

392 Poster LI. Second Messengers

IMMUNO-LOCALIZATION OF INOSITOL TRISPHOSPHATE RECEPTORS IN MOUSE VOMERONASAL OLFACTORY EPITHELIUM

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Previous studies have demonstrated that pheromones induce generation of the second messenger inositol trisphosphate (IP3) in mammalian vomeronasal organ (VNO), suggesting an important role for IP3 in vomeronasal signaling (Kroner et al., NeuroReport, 7:2989-2992, 1996; Wekesa and Anholt, Endocrinology, 138:3497-3504, 1997). Yet, it is not known whether IP3 activates IP3 receptors or other channels, such as TRP2 (Liman et al., Proc. Natl. Acad. Sci. USA, 96:5791-5796, 1999). Also it is not clear which types of IP3 receptors are expressed in VNO. We therefore examined their expression immunohistochemically by using sub-type specific antibodies directed against IP3 receptors type I, II and III. Thirty–five micron frozen sections of decalcified mouse nose epithelium containing VNO as well as cerebellum and kidney were obtained from mice perfused with buffered 4% paraformaldehyde. Immunoreactivity for type I and II IP3 receptor was observed in subsets of VNO neurons located mainly near the edges of the VNO. Interestingly, immunolabeling for type III IP3 receptor was detected in the apical portion of the epithelium as well as in axon bundles. These results suggest that IP3 receptors are heterogeneously distributed in mouse VNO sensory neurons.

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ODOR RECEPTOR GENE EXPRESSION AND G PROTEINS IN THE OLFACTORY SYSTEM OF THE GOLDFISH

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In rodents, microvillar vomeronasal receptor cells and ciliated receptor cells utilize different types of G proteins and odor receptor molecules. Goldfish olfactory epithelium has both ciliated and microvillar receptor cells contained together in the same epithelium. The aim of these experiments was to test whether a correlation exists between odor receptor molecules and G proteins in the various cells of the goldfish olfactory epithelium. We utilized fluorescent tyramide-amplified in situ hybridization to detect odorant receptor mRNA followed by indirect fluorescence immunocytochemistry for the different G proteins. Using confocal microscopy, we determined that Go co-localized with the V2R receptor molecules and Golf co-localized with the OR receptor molecules. These results suggest that in goldfish, like mammals, V2R-type receptors are associated with Go while OR receptors are associated with Golf. (Supported by NIH Grant DC03792 to John Caprio).

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IMMNOHISTOCHEMICAL AND ULTRASTRUCTURAL IDENTIFICATION OF G-PROTEINS IN THE OLFACTORY EPITHELIUM OF CATFISH

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In rodents, two olfactory receptor cell types (ORNs) are confined each to a separate compartment in the nose, and each cell type is equipped with a different G-protein alpha subunit; ciliated ORNs utilize Golf while microvillous receptor cells of the VNO utilize Go or Gi. Fish olfactory epithelium contains both ciliated and microvillar ORNs and a third receptor cell type, the crypt cell; all three ORN types are intermingled in the olfactory epithelium. We used antibodies to visualize Go and Golf in the catfish olfactory epithelium to test whether G protein expression correlates with ORN morphology. Cryosections and whole mount preparations of olfactory lamellae were processed immunohistochemically. Selected sections and whole mounts were postfixed in glutaraldehyde and OsO4 and embedded in plastic for electronmicroscopic examination . In catfish, the majority of Go-positive cells are located in the dorsal medial region of the lamellae. The quantity of ORNs labeled varies from one animal to the next and also between lamellae of one olfactory organ. On the contrary, Golf-positive cells are always abundant and spread evenly in the sensory area of the epithelium. Transmission electron micrographs reveal that the antibody to Go labels crypt cells. The reaction product is localized to the cytoplasm around the nucleus all over the cell body. Golf reactivity is present only in ciliated receptor cells. The reaction product is heavy in the cilia but is faintly visible in the cell membranes of the cell body but not within the cytoplasm. Supported by NIH Grant DC03792 (J. Caprio, P.I.).

395 Poster LI. Second Messengers

RECONSTITUTION OF AN OLFACTORY RECEPTOR-ACTIVATED cAMP SIGNALLING PATHWAY IN A MAMMALIAN CELL LINE

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Despite accumulating sequence data, little information is available on cognate odorant-receptor pairs and their EC_{50}-based odorant profiles. Functional expression of recombinant olfactory receptors (OR) in a mammalian cell line, has been achieved by N-terminal extension of OR with a rhodopsin tag and coupling to the IP_3 response-activating G proteins α15, 16 [1]. From initial screens, using single cell Ca^{2+}-imaging on 144 OR and 35 odorants, 2 mouse OR chimeras (I-C6, V-D2) emerged as responders to the odorant citronellal, with different EC_{50} values. Both OR share an amino acid identity of 38% over transmembrane regions II-VII. Testing a stable HEK-293 clone expressing recombinant Gα15 for coupling to endogenous β-adrenergic receptors and using a Ca^{2+} fluorescence imaging plate reader (FLIPR), we determined an EC_{50} value of 15±5.8 nM for Isoproterenol (ISO). In HEK-293-Gα15 cells, transfected with full-length OR I-C6 and V-D2, and stimulated with (-)citronellal, we determined EC_{50} values of 4.3 and 0.7±0.5 µM, respectively. To establish an olfactory-like signal transduction, we isolated a HeLa clone, expressing the olfactory cyclic nucleotide-gated homomeric α subunit (gift of Dr. Kaupp, Juelich) channel and connexin43. Using FLIPR, we found an EC_{50} value of 20±5 nM for ISO. Transfection of I-C6 or V-D2, and stimulation by (-)citronellal gave EC_{50} values of 4.7±3.5 and 1.8±0.9 µM, respectively. [1] Krautwurst, D. et al., Cell 95, 917-26 (1998).

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Wednesday April 25, 2001

Educational Outreach
GWIZ science center

12:00-3:30 Executive Committee
Executive Board Room

5:00-7:30 Registration
Prefunction Area

6:30 Opening Buffet
Salons A & B

Welcome & Awards

I. Givaudan Lecture
Salons C, D, E, & F

Social Gathering & Cash Bar
Prefunction Area

Organization meeting for students with travel awards
Salon G

Thursday April 26, 2001

Continental Breakfast 7:30-9:00

Slide Session
II. Gustatory Development
Salons E & F

Poster session
IV. Bitter Taste
V. Hedonics
VI. Spatial Temporal Processing in Olfaction
VII. Trigeminal Nerve
Salons A, B, H, & G

Symposium
III. Mechanisms of Differentiation and Migration of Progenitor Cells in the Olfactory System
Salons E & F

12:30-2:00 Minority and Clinical Travel Awardee Luncheon
Executive Board Room

3:30-5:00 NIH Workshop Funding Opportunities for the New Investigator
Salon E & F

5:00-7:00 Junior Investigator Cookout
Outdoors

Slide Session
VIII. Bitter Taste
Salons E & F

Poster session
X. Aging
XI. Assessment of Discrimination and Sensitivity
XII. Cell Differentiation in Development
XIII. Recognition of Chemosensory Signals
Salons A, B, H, & G

Symposium
IX. Genomics of Chemosensory Systems
Salons E & F

Friday April 27, 2001

Continental Breakfast 7:30-9:00

Slide Session
XIV. Chemosensory Transduction
Salons E & F

Poster session
XVI. Cognitive and Mood Effects in Olfaction
XVII. Modulation of Chemosensory Systems
XVIII. Psychophysics
XIX. Salt Taste
Salons A, B, H, & G

Symposium
XV. Functional Genomics in Neural Systems
Salons E & F

12:00-2:00 Business Meeting
Salons E & F

2:00-4:00 Softball Game
Fruitville and Lockbridge Rd

4:00-6:00 Workshop: Receptor Classification
Sarasota Room

5:00-7:00 Industrial Reception
Salons C & D

Slide Session
XX. Molecular Biology of Olfaction
Salons E & F

Poster session
XXII. Genetics and Genomics
XXIII. Gustatory Development
XXIV. Neurotrans.
XXV. Olf. Plasticity and Development
XXVI. Reproductive Odors
XXVII. Sweet Taste
Salons A, B, H, & G

Presidential Symposium
XXI. Seeing is Believing: Imaging Cellular Function in the Chemical Senses
Salons E & F

Saturday April 28, 2001

Continental Breakfast 7:30-9:00

Slide Session
XXVIII. Systems Analysis of Gustatory Function
Salons E & F

Poster session
XXX. Cortical Proc. in Chemosensory Systems
XXXI. Disease and Chemical Senses 1
XXXII. Olf. Develop.
XXXIII. Functional Studies Olf. Glom.
XXXIV. Transduction in Olf. and Vomeronasal Receptor Cells
XXXV. Orientation to Chemical Stimuli
Salons A, B, H, & G

Symposium
XXIX. Signal Transduction in the Vomeronasal Organ
Salons E & F

12:00-2:00 Clinical Luncheon
Salons C & D

4:30-6:00 Workshop
XXXVI. Molecular & Functional Mapping of the Olfactory Bulb
Salons C & D

Slide Session
XXXVII. Olfactory Processing
Salons E & F

Poster session
XXXIX. Develop. of Olfactory Glomeruli
XL. Perireceptor Processes
XLI. Receptors and Ligands
XLII. Taste Pathways
XLIII. Transduction in Sensory Cells
Salons A, B, H, & G

Symposium
XXXVIII. Odor/Taste Interactions and Flavor Perception
Salons E & F

Sunday April 29, 2001

Continental Breakfast 7:30-9:00

Slide Session
XLIV. Olfactory Cells and Pathways
Salons E & F

Poster session
XLVI. Disease and Chemical Senses 2
XLVII. Glomerular Organization
XLVIII. Multimodal Interactions
XLIX. Functional Characteristics of Sensory Cells
L. Taste Cells
LI. Second Messengers
Salons A, B, H, & G

Slide Session
XLV. Olfactory Development
Salons E & F

Locations

Slide and Symposium Sessions
Salons E & F
(All morning symposia begin at 10:00AM All evening symposia begin at 8:00 PM)

Poster Sessions & Exhibits
Salons A, B, H, & G

Continental Breakfast
7:30-9:00 Prefunction Area

MidMorning Break
9:30-10:00
Prefunction Area

Evening Break
Prefunction Area
7:45-8:15

Cash Lunch Bar
after morning sessions
Prefunction Area

DATES OF FUTURE MEETINGS

April 24-28, 2002
April 9 - 13, 2003