ACHEMS - 1994

ABSTRACTS

THE SIXTEENTH ANNUAL MEETING OF THE ASSOCIATION FOR CHEMORECEPTION SCIENCES

Hyatt, Sarasota
Florida
April 13-17, 1994

ACHEMS - 1993

Sixteen Annual Meeting
of the

Association for Chemoreception Sciences

ABSTRACTS

This book contains abstracts of the volunteer and invited papers and posters of ACHEMS 1994. Abstracts are listed in order of presentation at the meeting. The abstracts for slide presentations precede the abstracts for poster presentations which are scheduled concurrently. An author index is included. ***Also see the new summary by Topic and the List of Symposia and Special Events.***

PROGRAM COMMITTEE 1993-1994

TOPIC	*FORMAT*	*TIME*
Human Chemoreception I Trigeminal and Multimodal	Slides	Thurs. PM
Human Chemoreception II Gustatory	Posters	Thurs. PM
Human Chemoreception III Olfactory	Posters	Fri. PM
Human Chemoreception IV Trigeminal and Multimodal	Posters	Fri. AM
Human Chemoreception V Gustatory	Slides	Fri. PM
Human Chemoreception VI Olfaction	Slides	Sat. AM
Human Chemoreception VII Olfactory and Gustatory	Posters	Sat. PM
Human Chemoreception VIII Gustatory	Posters	Sun. AM
See also Animal Behavior II: for Human Infant Feeding (2)		
Sensory Evaluation and New Technology	Posters	Thurs. AM
Animal Behavior I	Slides (3)	Thurs. AM
Animal Behavior II	Posters	Thurs. AM
Animal Behavior III: Chemical Ecology	Posters	Sat. AM
Animal Behavior: Temporal Coding	Minisymposium	Fri. AM
Chemical Ecology: Stimuli	Posters	Sat. AM
Vomeronasal System	Posters	Thurs. AM
Trigeminal (Animal)	Posters	Fri. AM
Growth, Migration, Regeneration in Smell and Taste Systems	Slides	Thurs. AM
Olfactory Systems: Organization, Development Plasticity and Migration	Posters	Fri. PM
Taste Systems: Regeneration, Development, Evolution	Posters	Sun. AM
Olfactory Receptors Cells: Physiology to Specificity	Slides	Thurs. PM
Taste Cell Physiology	Slides	Fri. AM
Physiology & Transduction I (Smell and Taste)	Posters	Fri. PM
Anatomy/Physiology & Transduction II (Taste and Others)	Posters	Sat. AM
Physiology & Transduction III	Posters	Sat. PM
Physiology & Transduction IV	Slides	Sun. AM
Olfactory Receptors: Gene Organization	Slides	Sat. AM
Genetics of Chemoreception	Posters	Sun. AM
Nitric Oxide and Carbon Monoxide	Posters	Thurs. PM
Olfactory Central Pathways I	Posters	Thurs. PM
Olfactory Central PathwaysII	Slides	Fri. PM
Olfactory Central Pathways III	Posters	Sat. AM
Olfactory Central Pathways IV	Slides (3)	Sun. AM
Olfactory Central Pathways V	Posters	Sun. AM
Gustatory Central Pathways I	Posters	Thurs. PM
Gustatory Central Pathways II	Slides	Sun. AM

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SYMPOSIA AND SPECIAL EVENTS

Frito-Lay, Moskowitz Jacobs, Ajinomoto, and Kenji Nakanishi Awards	Wed. 8:00 PM
Givaudan-Roure Lecture	Wed. 8:30 PM
Symposium: Gene Expression in Neuronal Activity	Thurs. 10:50 AM
Clinical Luncheon *Tickets Required*	Thurs. 1:00 PM
Minisymposium/Workshop: Taste and Smell Phantoms and Distortions	Thurs. 3:30
Animal Behavior Minisymposium: Temporal Coding	Fri. 9:35 AM
Symposium: Spatial Coding: Molecules to Behavior	Fri. 10:45 AM
Grants Workshop	Fri. 1:00 PM
Minisymposium: Cell Lineage Analysis	Fri. 3:30 PM
Industry/Academia Buffet *Tickets Required*	Fri. 5:00 PM
Business Meeting *Dr. James Snow will address AChemS*	Sat. 11:45 AM
Education Outreach Workshop	Sat. 3:30 PM
Wine Tasting *Tickets Required*	Sat. 6:30 PM
IFF Award	Sat. 8:00 PM
IFF Symposium: Chemical Ecology	Sat. 8:15 PM

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CLINICAL LUNCHEON: FOCUS ON SMELL PHANTOMS

(Organizers: April Mott, CT Chemosensory Clinical Research Center and Jack Pearl, NIDCD)

Both clinical and basic scientists are invited to participate in the exploration of possible pathophysiologic mechanisms and proposed sites of intervention for phantom smells. Our featured guest will be a patient from Iowa, Darlene Herrick, who developed a smell phantom after head trauma. She is a two-time Pillsbury Bake-Off finalist and a winner of two other national recipe contests. Her participation will allow direct exposure of nonclinical chemosensory scientists to a patient with this intrusive disorder. After the case presentation, a panel consisting of April Mott, Donald Leopold (Johns Hopkins) and Nathan Zasler (National NeuroRehabilitation Consortium) will describe Mrs. Herrick's clinical findings, and provide a review of possible causes and management options for patients with phantom smells. Luncheon participants will be asked to assist in specific discussion areas: review of the clinical presentations of parosmias; determination of reasonable evaluation protocols; review of known therapies; proposed changes in evaluation and management; and development of possible avenues of future research.

SMELL AND TASTE PHANTOMS AND DISTORTIONS: MINI SYMPOSIUM AND WORKSHOP

Organizers: Jack Pearl, NIDCD and April Mott, CT Chemosensory Clinical Research Center; Moderator: Donald McBurney (University of Pittsburgh)

Studies indicate that chemosensory disorders are secondary to a variety of disease processes. Therefore, olfactory or gustatory complaints may serve as important presenting symptoms of a disease and may assist the physician in developing the differential diagnosis. In contrast to loss of smell function, major losses of taste function are rare. However, complaints of dysgeusia are as common as complaints of dysosmia. Approximately a third of the patients at the University of Pennsylvania chemosensory research center complained of dysgeusia. Patients may perceive distortions of smell or taste or chronic unpleasant sensations for which there is no obvious taste or smell stimulus. These dysosmias and dysgeusias, like tinnitus in audition, can be highly debilitating disorders. Although many cases are local in origin, that is, from substances present in the oral and nasal cavities, some may result from peripheral or central damage of taste nerves. Recognition of the origin of the unpleasant taste and smell sensations permits rational therapies. A workshop will be held on promising research directions to improve the diagnosis and management of dysosmia nd dysgeusia. Participants will include scientists from the NIDCD-supported clinical chemosensory research centers. Topics will include:

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Ultrastructure of Rat Circumvallate Taste Buds after Bilateral Denervation of the Glossopharyngeal Nerve. MICHAEL JACOBSON[1,2], TERRI A. SHERMAN-CROSBY[1,2], HILDEGARD CROWLEY[1,2], DEMETRIO QUISPE[1,2], HEIDI LENNOX[1,2], BRUCE OAKLEY[3] and JOHN C. KINNAMON[1,2]. (University of Denver, Denver, CO[1], the Rocky Mountain Taste and Smell Center, Denver, CO[2] and the University of Michigan, Ann Arbor, MI[3])

We are attempting to elucidate the determinants of synaptic structure in vertebrate taste buds by cross-reinnervating fungiform and circumvallate taste buds of the rat. As one of the control experiments for this study we are investigating the effects of denervation of circumvallate taste buds by bilateral section of the glossopharyngeal (IXth) nerve.

In the present study we have used a combination of light microscopy, transmission and scanning electron microscopy to examine the circumvallate taste buds of rats after denervation for comparison with cross-reinnervated taste buds. Our preliminary results confirm the work of others that suggest that only a few taste buds are present 3-4 weeks after denervation.

Our preliminary results fall into two classes. In most cases, no taste buds at all were observed in denervated papillae. In some papillae, however, a few to several taste buds were present. Thses taste buds appear normal with notable exceptions. Although nerve processes were present in these taste buds, the neural elements were fewer in number and smaller in diameter than in control animals. Currently we are investigating the origin of the innervation to those papillae with innervated taste buds.

This work was supported by NIH grants DC00244 and DC00285.

6

Salt Discrimination Before And After Rat Chorda Tympani Regeneration. STEVEN J. ST. JOHN, STACY MARKISON, and ALAN C. SPECTOR (Department of Psychology, University of Florida).

Anterior tongue taste buds degenerate following chorda tympani nerve (CT) section (CTX) and return after CT regeneration. In rats, CTX unambiguously disrupts performance on a presurgically conditioned sodium chloride (NaCl) vs. potassium chloride (KCl) discrimination task. Accordingly, we used the salt discrimination paradigm as a behavioral assay of the functional competence of the regenerated CT. Water-deprived rats were trained in a specially designed gustometer to maintain licking to either NaCl or KCl during 5 s trials (the S- salt) and to suppress licking to the other salt (the S+ salt). Rats failing to lick the S- salt after the first 2 s received a 30 s time-out; those failing to suppress licking the S+ salt received a mild foot shock. Three concentrations of each salt (0.05, 0.1, and 0.2 M) were used to make intensity an irrelevant cue. Training consisted of eleven 50 min sessions with the entire stimulus array. An additional 4 sessions served as the presurgical baseline. Seven rats then received bilateral CTX and 8 rats served as surgical controls. Three CTX rats and 4 control rats were tested over four sessions beginning 8 days after surgery (short-survival groups). The remaining rats were tested starting 49 or 50 days after surgery (long-survival groups). The CTX rats in the short-survival group were severely impaired in the salt discrimination task, replicating previous work. In contrast, the performance of all of the remaining rats was unequivocally normal. Post-mortem examination of tongues stained with methylene blue by an experimenter blind to the identity of the tissue revealed that the anterior tongues of CTX rats in the long-survival group contained a near-normal complement of taste pores, suggesting the CT had regenerated in these rats. The anterior tongues of rats in the short-survival group contained only a minimal number of pores. These findings demonstrate that salt discriminability is completely restored following regeneration of anterior tongue taste buds. The possibility that postsurgical central reorganization contributed to the recovery of function, however, cannot be ruled out.

Supported by PHS grants DC01628 and DC00161.

7

Effects of Chorda-Lingual Nerve Repair on Human Taste

J. ZUNIGA (Univ. North Carolina)
N. CHEN, C. PHILLIPS (Univ. North Carolina)

The purpose of this study was to determine if taste perception recovers and fungiform taste buds regenerate in humans following chorda-lingual nerve (C-L) repair. Eight volunteers with a transection injury of a unilateral C-L underwent citric acid taste tests and fungiform taste bud analysis on the anterior tongue prior to and 1, 3, and 6 months after repair. On both the repaired and uninjured sides of the anterior tongue, citric acid solutions were delivered to a closed, spatially-matched flow chamber. A 2-alternative forced choice staircase procedure was used to derive detection threshold. A cross-modal magnitude matching function for taste intensity judgement was obtained. The taste buds bathed by stimuli within the chamber were stained with methylene blue and recorded by videomicroscopy. The sip and spit method was used to contrast the spatially-matched condition with whole mouth stimulation. We determined that C-L transection results in decreased numbers of fungiform papillae and pores. Some papillae persist, but they were devoid of pores, smaller and paler, and some appeared cornified. All patients could detect and scale citric acid under whole mouth conditions and on the uninjured side of the anterior tongue before and after repair. Before repair they were devoid of taste perception on the side of injury. After repair fungiform papillae increased in size and density and re-established the normal ratio of pores per papilla. Some patients demonstrated an increase in the ability to detect and scale citric acid on the injured side over time. These preliminary studies suggest that fungiform taste buds regenerate and taste sensitivity recovers in adult humans following C-L repair.

Supported by NIDR DE10141

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The Vomeronasal System is Differentially Involved in Discrimination of Conspecific Odors in the Brazilian Short-tailed Opossum, *Monodelphis domestica*, Depending on Experience. RISA ROLAND (Midwood High School at Brooklyn College), LENA SHNAYDER, CHENG-SHU LI, AND MIMI HALPERN (SUNY Health Science Center at Brooklyn, Program in Neural and Behavioral Sciences, 450 Clarkson Ave., Brooklyn, NY 11203)

Previous work (Poran et al., 1993a; 1993b) has demonstrated that, in male opossums (*M. domestica*), nuzzling is a chemosensory behavior that brings biologically significant chemicals into the VNO and helps to identify familiar and non-familiar conspecifics. However, these experiments did not establish that a functioning vomeronasal system (VNS) is necessary for these behaviors. The goal of the present set of experiments was to determine the role of the opossum vomeronasal system in the nuzzling behavior. Two experiments were conducted, which differed in the extent of pre- and post-operative nuzzling experience that each animal received. Conspecific odors were collected on the surface of small glass vials either from the subject (familiar odor) or from a different animal (novel odor). The nuzzling test consisted of a 5-minute choice test in which each animal had an opportunity to nuzzle either the familiar or a novel stimulus. In the first experiment, each animal was allowed one pre- and one post-operative trial, whereas in the second experiment, each animal was tested four times both pre- and post-operatively. After the pre-operative testing, each animal was subjected either to vomeronasal nerve axotomy (VNX) or to sham surgery (CON). In the first experiment, where opossums had only limited nuzzling experience, VNX, but not the sham lesion, resulted in decreased nuzzling duration and loss of preference for the novel odor. Surprisingly, in the second experiment, where animals received more extensive behavioral testing prior to surgery, the same surgical procedures did not have the same effect. Both VNX and CON groups performed similarly post-operatively, retaining the differential nuzzling behavior to the novel stimulus. These data suggest that extensive pre-operative experience may attenuate the detrimental effects of VNX and point to the fact that learning, possibly association of olfactory cues with vomeronasally-mediated signals, may be taking place during the pre-operative nuzzling test.

9

Exposure of Mice to Androstenone Induces Behavioral Sensitivity to Androstenone. VERA V. VOZNESSENSKAYA (A.N. Severtzov Institute of Evolutionary Animal Morphology and Ecology, Moscow, Russia) and CHARLES J. WYSOCKI (Monell Chemical Senses Center, Philadelphia, PA)*

Previously, using a conditioned aversion paradigm, we noted that genetically uniform NZB/B1NJ (NZB) mice appeared to be insensitive to androstenone (AND) and that CBA/J (CBA) mice could readily detect the odorant. We now have obtained estimates of sensitivity to AND, both before and after exposure to the compound, from 9 ♂ NZB and 8 ♂ CBA mice who were trained to run to the odorant-scented arm of a Y-maze for water reward. AND thresholds were estimated after mice reached a criterion of 80% correct choices in mixed trials of amyl acetate, phenylethyl alcohol or Galaxolide®. Thresholds were obtained for 6 of the NZB mice: With 70-100% accuracy they could detect an air-stream flowing over 0.1% (w/v) AND in mineral oil, but not 0.05% AND. Although no thresholds were obtained for 3 NZB mice, subjective observations suggested that they could detect AND crystals. All of the CBA mice could detect AND at a concentration 2000-fold more dilute, i.e., $5x10^{-5}$%, but not 4000-fold. The mice were then exposed to 0.1% AND in oil for 14 days, 16 hours per day. AND thresholds were once again estimated. With 80-100% accuracy, thresholds for the NZB mice ranged from $3.125x10^{-3}$% (n=2; one of these mice could not detect 0.1% AND before exposure) to $2.5x10^{-2}$% (n=1; this mouse could not detect 0.1% AND before exposure) with the majority having a threshold at $6.25x10^{-3}$%. AND thresholds for the CBA mice decreased 100-fold; they were uniform at $5x10^{-7}$%. Interestingly, EOGs obtained from AND-exposed NZB mice revealed sensitization (the EOG magnitude is increased) but EOGs from CBA mice were unaffected by exposure to AND (Wang, Wysocki and Gold, Science, 1993, 260:998). The results obtained from these two approaches have yet to be reconciled.

*Supported in part by NIH Grant DC00298 and USDI, USFWS, Office of International Affairs.

10

Olfactory Responsiveness of Female Goldfish to Sex Pheromones is Enhanced by Exposure to Elevated Levels of Circulating Androgenic Sex Hormones. P. W. SORENSEN and L. BOWDIN (Dept. of Fisheries & Wildlife, Univ. of Minnesota)

Recent studies have demonstrated that as measured by electro-olfactogram (EOG) recording, the olfactory epithelium of mature male goldfish is more sensitive to F prostaglandin-derived sex pheromones than is that of female goldfish. Additionally, only mature males respond behaviorally to water-borne F prostaglandins. To determine whether this sexual dimorphism is associated with differences in circulating androgenic hormones female goldfish were implanted with silastic pellets containing either oil (control) or 11-ketotestosterone (11-KT), the presumed androgenic sex hormone in teleost fish. Three weeks later, these fish were placed into tanks with sexually-active females releasing pheromones to determine whether they had acquired male behavioral attributes. Five of six 11-KT treated fish exhibited male spawning behavior while none of the control fish responded. Following this test, the peripheral olfactory sensitivity of these fish to a variety of pheromonal and non-pheromonal stimuli was characterized by EOG recording. EOG recording demonstrated that 11-KT treated fish were more sensitive than control fish (had larger relative EOG responses) to the following 4 sex pheromones: prostaglandin $F_{2\alpha}$, 15-keto-prostaglandin $F_{2\alpha}$, 17α,20β-dihydroxyprogesterone, and 17α,20β-dihydroxyprogesterone-20-sulfate. Furthermore, EOG responsiveness of 11-KT and control fish to L-serine, taurolithocholic acid sulfate (a bile salt), and androstendione did not differ suggesting that endocrine influences on peripheral olfactory function are specific. However, neither the olfactory sensitivity, nor the behavioral responsiveness of 11KT-treated females was on par with mature males suggesting that although short-term exposure to steroid hormones modifies olfactory function, the process is complex and takes some time.

P.W.S. was supported by NSF:BNS9109027.

11

Immediate-Early Genes and Stimulus-Transcription Coupling in the Nervous System. MICHAEL D. HAYWARD, SHU-CHENG CHEN, TOM CURRAN and JAMES I. MORGAN (Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110).

Cellular immediate-early genes (cIEGs) are rapidly and transiently induced by a diverse array of extracellular stimuli that includes mitogens, growth factors and neurotransmitters. Since many of the cIEGs encode transcription factors they are thought to serve as nuclear third messengers that couple extracellular stimuli to long-term alterations in cellular phenotype by changing target gene expression. We have been analysing the expression of two prototypic immediate-early genes, c-*fos* and c-*jun*, in mice harboring *fos-lac*Z and *jun-lac*Z transgenes. While studies in cell culture have shown these genes to have very low basal levels and to be transiently induced by stimuli, analysis of the transgenic mice has revealed sites of continuous expression of both *fos* and *jun*. Interestingly these sites are rarely coincident, despite the fact that *fos* and *jun* are coinduced under most conditions in culture and are thought to heterodimerize with one another to form the transcription factor, AP-1. Thus in vivo, Fos and Jun presumably interact with other partners, and the simplistic notions derived from cell culture do not obtain in the intact organism. Many of the cells that express these genes in vivo seem to be undergoing critical developmental steps such as trans- and terminal differentiation, migration and even death. We suppose that growth factors may be underlying some these inductive events. This notion will be examined with special reference to the olfactory system.

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Activity-induced IEG Expression: What Does it Mean for the Cell? STEVEN E. HYMAN, CHRISTINE KONRADI, REBECCA COLE, BARRY E. KOSOFSKY (Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital, Harvard Medical School, Boston, MA)

The indirect dopamine agonists, amphetamine and cocaine have been shown to induce expression of immediate early genes (IEGs), such as *C-fos* in rat striatum. It has been hypothesized that *c-fos* may couple D1 dopamine receptor activation to induction of target genes, such as the endogenous opioid gene encoding prodynorphin, or alternatively that these genes may be regulated in parallel. To determine whether *c-fos* was activated in the same cells as their putative targets, we used double *in situ* hybridization. We find that cocaine activates *c-fos* in substance P/dynorphin neurons and not in enkephalin neurons. We have previously shown in a different stimulus paradigm that in striatum CREB protein interacts and Fos protein does not interact with the second messenger-responsive enhancer within the proenkephalin gene. Thus we investigated the binding of CREB and Fos to regulatory regions within the prodynorphin gene and within the *c-fos* gene itself. We find that CREB interacts with sequences in both the prodynorphin and *c-fos* genes that confer responsiveness to dopamine. We also find that amphetamine induces CREB phosphorylation, and therefore, possibly, CREB-mediated transcriptional activation in striatum *in vivo*. In primary striatal cultures we find by gel shift assay that dopamine induces phosphoCREB binding to regulatory elements within the prodynorphin and *c-fos* genes. Finally we show by antisense oligonucleotide injection that CREB is required for amphetamine induction of *c-fos* in striatum. The data are consistent with a model in which dopamine induces *c-fos* and prodynorphin gene expression in striatum via transcription factor CREB. We conclude that the specificity and biological relevance of stimulus-induced transcription factor activation can be investigated in brain.

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Regulation of FOS/JUN (AP/1)-Activity and AP-1-Dependent Gene Expression in Vivo and in Vitro. SABINE GACK, INGRID HERR, HANS VAN DAM, THOMAS OEHLER, BERND BAUMANN, ULRICH RÜTHER*, PETER ANGEL Kernforschungszentrum Karlsruhe, Institut für Genetik, Karlsruhe, FRG; and *Medizinische Hochschule Hannover, Institut für Molekularbiologie, Hannover, FRG.

cJun and cFos are the major components of the sequence-specific transcription factor AP-1, which regulates specific gene expression in response to growth factors, cytokines, tumor promoters, carcinogens and expression of certain oncogenes. In tissue culture cells post-translational modification, including hyperphosphorylation of pre-existing cJun (and cFos), as well as mutual interference with cellular and viral proteins seem to initiate the subsequent transcriptional activation of the c-Jun gene itself. Enhanced cJun (and cFos) protein synthesis is required for a long-lasting induction of AP-1-dependent target genes, e.g. collagenase, which is mediated, at least in part, by enhanced occupation of the AP-1 binding site in the collagenous promoter by Jun/Fos Immediate activation of the c-jun promoter by TPA, UV irradiation or the E1A product of Adenovirus is mediated through pre-bound heterodimers containing cJun and ATF (a member of the CREB/ATF family) binding to two AP-1-like binding sites (Jun-1, Jun-2). To mediate its transcriptional activation potential Jun requires the presence of a specific co-factor (p52/54) that interacts with the transactivation domain of Jun (depending on the phosphorylation status of Jun) to link Jun and components of the basal transcriptional machinery. We have chosen transgenic mice that either overexpress cFos, or mice that lack cFos expression to study the role of AP-1 in an in vivo relevant multicellular system. The pathological consequences as well as the effects on tissue-specific alterations in gene expression will be discussed.

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Neurotransmitter Control of Gene Expression.
JEAN M. LAUDER (Sch. of Medicine, University of N.C. at Chapel Hill)

Neurotransmitters act as growth regulatory signals during neural development and serve similar functions in primitve organisms and early embryos. These functions may be reinitiated during responses of the brain to injury. In their capacities as growth regulatory signals, neurotransmitters appear to utilize the same receptors and signal transduction pathways mediating their actions in the adult nervous system. This raises the possibility that the specialized role of neurotransmitters in the vertebrate nervous system may have evolved from more primitive functions in lower phyla, and that these functions are reiterated during development and plasticity. Due to the repertoires of developing cells, encounters with neurotransmitters can result in regulation of such processes as cell proliferation, migration, differentiation, metamorphosis, morphogenesis, synaptogenesis, or receptor expression. Many of these effects may involve altered gene expression resulting from activation of second messengers, immediate early genes or other transcription factors. Several examples of neurotransmitter regulation of gene expression will be discussed: 1) effects of serotonin (5-HT) receptor ligands on craniofacial morphogenesis and expression of S-100ß, tenascin and cartilage proteoglycan core protein; 2) effects of 5-HT receptor ligands on cAMP formation and expression of S-100ß in embryonic glial cells; 3) ligand regulation of developing 5-HT and GABA receptors; and 4) trophic effects of GABA receptor ligands on embryonic brainstem neurons.

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Functional mapping of odor-activated neurons in olfactory bulb. KATHLEEN GUTHRIE and CHRISTINE GALL (University of California, Irvine, CA 92717)

The protein products of the cellular immediate-early genes c-fos and c-jun comprise the transcriptional regulatory factor known as AP-1. A few years ago, the demonstration that neural activity, initiated by a variety of stimuli, induces the expression of these genes by neurons, elucidated a specific cellular mechanism for activity-dependent regulation of neuronal gene expression (Morgan et al., 1987; Sagar et al., 1988). Since that time, induction of immmediate-early gene expression, and in particular c-*fos* expression, has been used to map activity in a wide variety of CNS systems with two major goals in mind. The first makes use of the resolution provided by cellular localization of c-*fos* mRNA or Fos protein to identify those neurons activated, directly and transynaptically, by a particular stimulus. The second is to identify both the cascade of cellular events which mediate c-fos induction, as well as identifying the specific target genes subject to c-*fos* regulation.

Our laboratory has used changes in the expression of the c-*fos* gene to identify and map the distribution of main olfactory bulb neurons activated by odor stimulation under a variety of conditions. We find that brief odor presentation to awake rats coordinately activates neurons in discrete regions of the glomerular layer and in underlying portions of the external plexiform, mitral and granule cell layers. The translaminar pattern of cells expressing c-*fos* corresponds to the "functional unit" predicted by the synaptic organization of the bulb, and the distribution of these activated units is topographic with regard to the odor stimulus. The appearence of this pattern of activity is altered by changes in the intensity and duration of the odor stimulus and by the administration of pharmacological agents. The cellular pattern of c-*fos* expression also displays distinct differences over the course of postnatal development. One of our long term goals is to identify potential target genes of c-*fos* regulation in olfactory bulb neurons, and toward this end we have begun colocalization studies to better characterize the phenotypes of odor-responsive cells.

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Dysosmia Among Patients at the UCSD Nasal Dysfunction Clinic: Etiology and Effect on Olfactory Function. CARLO QUINONEZ (San Diego State University), TERENCE M. DAVIDSON, ALFREDO A. JALOWAYSKI (UCSD Medical Center) STEVEN NORDIN and CLAIRE MURPHY (UCSD Medical Center and San Diego State University)

Dysosmia, including both parosmia and phantosmia, is a condition wherein the sense of smell becomes distorted, or when a person smells an odor for which there is no stimulus, respectively. This study examines the incidence rates of complaints of parosmia and phantosmia reported on questionnaires completed by patients at the UCSD Nasal Dysfunction Clinic within the last six years. The patients were diagnosed by means of CT scans, nasal physiology, nasal cytology, and endoscopic examination, and subsequently classified into the following categories: post-viral upper respiratory infection (URI), head trauma, sinusitis, allergic rhinitis, toxic exposure, deviated septum, psychiatric problems, and presbyosmia (ten subjects fell into more than one category). Olfactory thresholds were obtained for the odorant butanol using the two-alternative, forced choice ascending method. For the odor identification task subjects were present with seven common everyday odors and then asked to identify them from a list of names which included ten distractors. Among the patients who reported dysosmia, the largest numbers fell into the categories of post-viral URI, allergic rhinitis, sinusitis, and head trauma. An ANOVA was performed on the butanol threshold and odor identification scores. Of particular interest is the significant interaction between the subjects' complaints of dysosmia and the type of olfactory test: for subjects who reported dysosmia, the odor identification score was lower. In addition, the division between the odor identification and detection performance was greater than that for subjects who reported no dysosmia. The findings suggest that not only is dysosmia reasonably common in post-viral URI, allergic rhinitis, and sinusitis, but also that dysosmia affects odor identification performance more than odor detection sensitivity.

Supported by NIH grants AG04085 (CM) and Training Grant DC00032 (SN).

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Neurorehabilitative Assessment and Treatment of Post-Traumatic Olfactory Dysfunction. NATHAN D. ZASLER (National NeuroRehabilitation Consortium) and RICHARD M. COSTANZO (Virginia Commonwealth University).

Traumatic brain injury (TBI) affects the lives of over 2 million Americans each year. Between 50 to 70 thousand individuals are left with significant long term functional disability. Olfactory dysfunction including sensory distortions (parosmia and phantosmia) are often over-looked neurologic impairments resulting from TBI. The differential diagnosis of post-traumatic focal neurologic findings of anosmia and dysosmia includes: tearing of the olfactory nerve(s), nasal bone fractures, focal parenchymal brain contusions, skull fractures with associated rhinorrhea, temporolimbic epilepsy and drug induced olfactory dysfunction. Diagnostic assessment should include standard chemosensory batteries as well as appropriate neurodiagnostic tests. After defining the olfactory impairment, the clinician should delineate the resultant functional disability, if any. An adequate history is critical in order to assess behavioral changes, vocational and avocational issues, and the impact on activities of daily living. "Quality of life" issues are seldom addressed adequately by most clinicians working with this patient population; therefore, attention is often not given to compensatory strategies geared at minimizing these losses. An array of rehabilitative compensatory strategies will be reviewed. The role of patient education, support, and general rehabilitation approaches will be discussed. Differences in impairment rating (utilizing the American Medical Association Guidelines) and functional disability evaluation will also be reviewed with particular attention to the medicolegal context.

Supported by NIH grant DC00165 (RMC)

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Effects of Topical Anesthesia on Dysgeusia and Burning Mouth. JAMES ANDELIN, APRIL E. MOTT and MARION E. FRANK (University of Connecticut Health Center, Farmington, CT, USA).

The neurological causes of dysgeusia and burning mouth are unknown. We utilized an approach suggested by Bartoshuk (1989), topical anesthesia, to address the origin of these symptoms. Patients were asked to rate the intensity of their symptoms on a scale from 0 (none) to 9 (very strong) before and following oral topical anesthesia (dyclonine: 0.5-1.0%, dyclonine plus Benadryl, or xylocaine). We obtained data on 71 patients: 70 reported dysgeusias (5 also reported burning) and 1 reported burning alone. The dysgeusias were either persistent phantoms of sweet, salty, sour, bitter, and/or metallic qualities (63 including 5 with burning); or atypical taste qualities (7 patients). The Table gives the results; symptoms were either abolished (Abol), reduced (Redu), increased (Incr), unchanged (Unch) or changed in a complex way (Comp). To change in a complex way, the symptom's intensity would alternately increase and decrease after anesthesia.

TABLE: EFFECTS OF TOPICAL ANESTHESIA ON ORAL SYMPTOMS

Symptom:	Numbers of Patients					
	Abol	Redu	Incr	Unch	Comp	Total
Dysgeusia						
Typical	34	18	2	3	1	58
Atypical	6	1	0	0	0	7
with Burning	2	2	0	1	0	5
Burning						
Alone	0	0	1	0	0	1
with Dysgeusia	1	0	3	0	1	5

In 90% of our patients, all types of dysgeusia were lessened with topical anesthesia, suggesting a symptom triggered by peripheral neural excitation via spontaneous activation of the nerve fibers or excitation of the taste buds by a taste stimulus present in the mouth. In contrast, albeit few cases were studied, 4 out of 6 of our oral burning patients noted an increase in the symptom's intensity following anesthesia. This suggests peripheral excitation may be inhibiting the burning symptom.

[Supported by NIH grant P50 DC00168]

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Taste Phantoms: Diagnosis Via Topical Anesthetics. L.M. BARTOSHUK, V.B. DUFFY, J. KVETON (Yale University School of Medicine), F. CATALANOTTO (University of Medicine and Dentistry of New Jersey), J. WEIFFENBACH (National Institute of Dental Research).

Inhibitory interactions between the central projection areas of the chorda tympani (VII) and glossopharyngeal (IX) taste nerves (Halpern and Nelson, 1965) suggest possible mechanisms for two types of taste phantoms. We have described phantoms in the area innervated by IX associated with anesthesia of (or injury to) contralateral VII. Since these phantoms are abolished by topical anesthesia of the mouth, we suggest that they may result when spontaneous activity in IX is interpreted as a signal once inhibition from VII ceases. Thus we call them release-of-inhibition phantoms. The second type of phantom occurred in five patients who suffered injuries to taste nerves. VII was severed in four patients leading to a salty phantom and IX was severed in the fifth patient leading to a bitter phantom. All five phantoms intensified with topical anesthesia of the mouth. Since these phantoms apparently result from some yet to be identified stimulation of the damaged nerve generated at the site of injury, the intensification after anesthesia of the mouth reflects the abolition of the inhibition normally exerted by intact nerves. We call these nerve-stimulation phantoms. When patients present with chronic tastes, the first aim should be to determine whether or not they are tasting some substance that is actually in the mouth. If not, then a spatial taste test can be administered to determine whether or not any taste nerves are damaged and the mouth can be anesthetized to determine whether the phantom will be abolished or intensified. A damaged nerve with a contralateral phantom that is abolished with anesthesia suggests a release-of-inhibition phantom. A damaged nerve with an ipsilateral phantom that intensifies with anesthesia suggests a nerve-stimulation phantom.

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Membrane Currents in Mammalian Olfactory Epithelium and Vomeronasal Organ Receptor Cells
BRUCE W. MURROW (University of Colorado Health Sciences Center). BRUCE W. JAFEK (University of Colorado Health Sciences Center).

A comparison of membrane properties between mammalian olfactory epithelium (OE) and vomeronasal organ (VNO) receptor cells has been initiated using the whole-cell voltage-clamp technique. Isolated receptor cells from rat, mouse, hamster, and human were identified by their morphology. In general, VNO receptor cells (soma diameter 12 ± 1 μm [mean ± SD]; dendrite length 57 ± 20 μm, n=5) were larger than those from the OE (soma diameter 7.5 ± 1 μm; dendrite length 35 ± 13 μm, n=5). Both receptor types expressed an array of voltage-dependant ion currents, including various types of I_K and an inactivating, TTX-susceptible inward current with characteristics like I_{Na}. Nearly every cell had a transient inward current that activated most often at a threshold between -58 mV and -68 mV (VNO) or between -68 mV and -78 mV (OE); peak inward current was reached usually by -10 mV. In both OE and VNO receptors, the voltage-dependance of inactivation for the inward current was markedly negative with respect to that in other types of neurons (VNO: $V_{1/2}$= -105 ± 13 mV, n=5; OE: $V_{1/2}$= -112 ± 11 mV, n=5). The maximal zero-current voltage measured was -73 mV and -58 mV for VNO and OE receptors, respectively. Even at these maximal measured zero-current voltages, little I_{Na} would be available to the receptor cells. As has been suggested with OE receptors (Rajendra S., Lynch J.W., and Barry P.H., Pflugers Arch., 1992, 420: 342-346), perhaps the "true" resting membrane potential in mammalian VNO receptors is substantially more hyperpolarized than that measured. Alternatively, the experimental process may result in a shift in the voltage-dependency of I_{Na} inactivation. Studies on isolated human OE receptor cells also revealed inward and outward voltage-dependent currents. Three of four cells exhibited an inward current with characteristics like I_{Na}. As in other mammalian nasal chemosensory cells, the voltage-dependant inactivation of this current was relatively hyperpolarized with a $V_{1/2}$ near -110 mV.

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Studies on Vomeronasal (VN) Bipolar Neurons

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Isolated sensory neurons of the VN organ were studied with a retrograde marker, and under tight-seal, whole-cell recording. Cells were dissociated from mouse and rat VN organs after exposure *in situ* to low-Ca^{2+} saline with Subtilopeptidase-BPN' (1.5 mg/ml) and urea (0.3 M). Initial studies were performed using fluorescent latex microspheres (0.03 um). After injection of the microspheres in the accessory olfactory bulb, cells of the VN organ were dissociated and the fluorescent beads were localized in a subset of neurons. Fluorescent labeled cells possessed an oval cell body with distinct axonal and dendritic processes. These bipolar cells also stained positive for neuron-specific enolase. Under whole-cell clamp mode, basic membrane properties and voltage-dependent currents were studied in bipolar neurons. In current-clamp, the resting membrane potential was measured at -50 to -60 mV; some sensory neurons displayed spontaneously firing of action potentials. In voltage-clamp, transient inward currents were activated near -30 mV and blocked with tetrodotoxin. Sustained outward currents were activated with more depolarizing pulses and blocked with tetraethylammonium. Finally studies were performed under whole-cell recording to determine the currents underlying sensory transduction in VN receptor neurons. N-amyl-acetate, KCl, bath solution and pheromones (2-(*sec*-butyl)-4, 5-dihydrothiazole; dehydro-*exo*-brevicomin; & lactol) were applied for 10 msec duration via a puffer pipette placed within 10-20 μm of the bipolar neurons. Pulses of KCl directed at the soma initiated inward depolarizing currents (150-450 pA), while exposure to the bath solution directed at the soma and dendrite had no observable effect. Pulses of N-amyl-acetate directed at the dendrite induced inward depolarizing currents (150-350 pA), while pheromones directed at the dendrite elicited outward hyperpolarizing currents (150-500 pA). These results indicate that some VN sensory neurons respond differentially to pheromones and general odorants.

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Vomeronasal Mucosa Expresses Different Sugar Residues in Certain Glycoconjugates than the Olfactory and Septal Mucosae.

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The localization of α-D-galactose (α-Gal), α-*N*-acetylgalactosamine (α-GalNAc), β-GalNAc, and *N*-acetylglucosamine (GlcNAc) sugar residues was examined in the vomeronasal organ (VNO), olfactory mucosa (OM), and septal organ of Masera (SO) of six-week-old Sprague-Dawley rats with lectin histochemical techniques. Six lectinoprobes were used: *Bandeirae simplicifolia* I isolectin B_4 (BS-I-B_4) for detecting terminal α-Gal, *Dolichos biflorus* agglutinin (DBA) for α-GalNAc, *Vicia villosa* agglutinin (VVA) and *Vicia villosa* agglutinin isolectin B_4 (VV-B_4) for terminal β-GalNAc, succinyl wheat germ agglutinin (S-WGA) for terminal and internal GlcNAc, and *Datura stramonium* agglutinin (DSA) for internal GlcNAc residues. Intense reactivity for BS-I-B_4, VVA, and VV-B_4 was observed in the mucosensory compartment of the VNO, as was moderate punctate labeling of vomeronasal receptor neurons. In contrast, binding sites for these lectinoprobes were not observed in the mucosensory compartment of either the OM or SO. Binding sites for S-WGA and DSA were observed in the mucosensory compartment and receptor neurons of the VNO as well as the OM, and SO. Granular labeling by S-WGA was observed primarily in the supranuclear region of the receptor neurons, while DSA primarily labeled the plasmalemma. Binding sites for DBA were observed in the mucosensory compartment of the VNO, but not within the vomeronasal sensory epithelium, OM, or SO, suggesting the presence of terminal α-GalNAc sugar residues in the mucoid component of the compartment and/or vomeronasal dendritc terminals. Secretory granules in acinar cells of vomeronasal glands (VNG) and posterior glands of the nasal septum (PGNS) contained binding sites for all lectinoprobes, whereas those in the Bowman's glands in the OM and glands of the SO contained binding sites for only DSA. Our study indicates that glycoconjugates in the vomeronasal receptor neurons, VNG, and PGNS differ from those in the OM and SO in that terminal α-Gal and GalNAc sugar residues are expressed only in vomeronasal receptor neurons and secretory glycoconjugates in the VNG and PGNS, suggesting that certain glycosyltransferases are only expressed in these neurons and the acinar cells. Supported by NIH-NIDCD-00159 (TVG) and -01715 (MLG).

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Localization of Neutrophils in the Nonsensory Epithelium of the Vomeronasal Organ in Virus-Antibody-Free Rats.

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The presence of β_2-microglobulin (β_2-m)- and immunoglobulin-E (IgE)-immunoreactive cells selectively in the nonsensory epithelium (NE) of the vomeronasal organ (VNO) of virus-antibody-free rats has been reported previously (Getchell et al., '93, Chem. Senses, 18; 560). They have been identified unambiguously as neutrophils by their immunolabelling with lactoferrin. Numerous neutrophils were localized in the NE and its lamina propria (LP), and a few adhered to the endothelium of the cavernous body (CB). However, tissue damage normally accompanying neutrophil infiltration was not observed. In order to investigate adhesion and chemotactic factors associated with infiltration of neutrophils, and to investigate a possible factor preventing tissue damage in the NE, VNO sections were stained with antibodies to leukocyte function-associated antigen (LFA-1: CD11a/ CD18), a β2 integrin; interleukin-8 (IL-8), a neutrophil attractant; and manganese (Mn)- and copper-zinc (Cu-Zn) superoxide dismutase (SOD), free-radical scavengers. Neutrophils expressed intense CD18 immunoreactivity intracellularly and on their surfaces. Intense IL-8 immunoreactivity was visualized in the granules of the mast cells in the LP, the vomeronasal glands (VNGs), and the mucus covering the NE but not the sensory epithelium. Intense MnSOD and CuZnSOD immunoreactivity was evident in columnar cells of the NE and in VNGs. The infiltration of neutrophils may result from repeated ischemia-reperfusion stress caused by dilation and constriction of the CB during functioning of vomeronasal pump. Neutrophils adhere to the CB when CD18 binds to its ligand on the CB endothelium. IL8 may act as a chemotactic stimulus. Dismutation of neutrophil-generated free radicals by SODs may prevent tissue damage in the NE.

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VN Chemoattractant Receptors in Garter Snakes Are Coupled to Multiple G proteins

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Vomeronasal (VN) chemoattractant signal transduction in garter snakes appears to be mediated by a G protein-receptor linked process. We have taken advantage of the fact that pertussis toxin (PTX) preferentially catalyzes α subunits of PTX-susceptable G proteins in a heterotrimeric form. We have shown that both $G_{i\alpha}$ and $G_{o\alpha}$ subunits are substrates for ADP-ribosylation by PTX in the garter snake VN epithelium. ES20, a chemoattractive glycoprotein purified from electric-shock induced earthworm secretion, causes dissociation of 41 kDa G_α subunits from G protein heterotrimers as indicated by reduction of PTX-catalyzed ADP-ribosylation of VN epithelial membranes in a ES20 concentration-dependent manner. G protein activators, such as GTPγS, Gpp(NH)p and AlF_3, mimic the effect of ES20. GDPβS, a G protein inactivator, antagonizes the ES20-induced attenuation of the 41 kDa ADP-ribosylation product. Addition of ES20 to VN membrane preparations decreases cAMP levels whether stimulated by GTPγS or forskolin or in the absence of exogenous stimulators. In contrast, ES20 binding increases IP_3 production about 7.5 fold compared to IP_3 levels in the absence of ES20. This stimulatory effect of ES20 is enhanced by the addition of GTPγS. Taken together, these results suggest that several classes of G proteins may be involved in ES20 signal transduction. Considering that the expression of G_o protein is restricted to the VN sensory epithelium, whereas G_i protein is found in both sensory and non-sensory epithelia, phosphotidylinositol turnover may play a dominant role in ES20 signal transduction.

This work was supported by NIH grant DC 00104-18.

Vapor vs. Liquid Delivery of Chemoattractants to the Olfactory and Vomeronasal Epithelia Results in Different Effects on the Firing Activity of Mitral Cells in the Main and Accessory Olfactory Bulbs of Garter Snakes. CHENG-SHU LI and MIMI HALPERN (SUNY Health Science Center at Brooklyn, Department of Anatomy and Cell Biology, 450 Clarkson Ave., Brooklyn, NY 11203)

We previously demonstrated that in garter snakes mitral cells of the accessory olfactory bulb increase their spontaneous firing, in a concentration-dependent manner, when either earthworm wash, goldfish wash, or the B_2 protein (a protein purified from earthworm wash) were delivered to the vomeronasal (VN) epithelium (Li et al., *Soc. for Neurosc. Abstr.*, 1993). In the present study, we compared the relative effectiveness of vapor delivery vs. liquid delivery of either earthworm or goldfish wash to the VN and main olfactory (MO) epithelium on the firing activity of mitral cells in the main (MOB) and accessory (AOB)olfactory bulb. Airborne delivery of earthworm wash and goldfish wash to the MO epithelium increased spontaneous firing of mitral cells in the MOB, although there was no significant effect of these chemoattractants, delivered in liquid form to the MO epithelium, on the firing activity of the MOB mitral cells. On the other hand, liquid delivery of earthworm and goldfish wash to the VN epithelium increased the spontaneous firing of mitral cells in the AOB, but vapor delivery to the VN epithelium had no effect. These results demonstrate in garter snakes that the MO epithelium is optimally stimulated by airborne odorants and the VN epithelium is optimally stimulated by odorants in a liquid state.

Brain *c-fos* expression following electrical stimulation of the vomeronasal organ. GWEN FERNANDEZ-FEWELL and MICHAEL MEREDITH. (Program in Neuroscience, Florida State University, Tallahassee FL 32306-4075 USA

c-fos expression in the forebrain was monitored following electrical stimulation of the vomeronasal organ (VNO) to investigate potential brain activation by vomeronasal sensory input. Electrodes were implanted unilaterally via a midline incision in the palate, in pentobartibal anesthetized male hamsters. Two 125 um teflon coated wires were bared 0.5 mm at the tip andeach inserted approx. 1 mm, into one of two burr holes, one near the anterior end, one midway along the vomeronasal capsule. They lay between the capsule and organ so that current between them would pass largely through the VNO. The wires were sealed in place with cyanoacrylate tissue adhesive and their free ends passed under the skin around the nose then along the dorsal skull surface to a connector mounted on the parietal bone. The palate was sutured and the animals allowed 3 days to recover. A flexible cable connected with a constant current stimulator. Animals were either stimulated for 45 min intermittently with low current in their home cages, or were connected but not stimulated for 45 min. After an additional 45 min disconnected from the stimulator, they were overdosed with pentobarbital, perfused with saline and 4% paraformaldehyde. Current was reduced if there was any sign of stimulus driven movements. In some cases bipolar recording electrodes were implanted at the dorsal border of the posteromedial cortical amygdala, ipselateral to the stimulating electrodes, to monitor the remotely recorded amygdaloid field potential (Meredith & Licht 1987), so that successful VNO stimulation could be assured. Effective pulse currents were within the range that produced no stimulus driven movements in the awake animal. Typical stimulation currents were 120-175 uA (0.5-1 ms pulses). Low level Fos activation occured in accessory olfactory bulb and amygdala, and in the anterior medial preoptic/ diagonal band region. In the latter, a few LHRH immunoreactive (ir) cells were also Fos-ir, a colocalization never seen in male hamsters following mating behavior or stimulation with female chemical stimuli. Whether this represents activation of the Nervus terminalis system by VNO stimulation is not yet clear. Experiments continue. Supported by NIH grant DC 00406.

Studies of Compartmentalization in the Developing Accessory Olfactory System. G. A. SCHWARTING, K. YOSHIDA and J.E. CRANDALL. The Shriver Center, Waltham, MA 02254 and Neuroscience Program, Harvard Med. Sch., Boston, MA 02115.

CC1 and CC6 are monoclonal antibodies that recognize subsets of vomeronasal neurons. Axons from $CC1^+$ neurons terminate in the rostral accessory olfactory bulb (AOB), whereas axons from $CC6^+$ neurons terminate in caudal glomeruli of the AOB but are also present in the nerve layer of the rostral AOB. These data suggest that molecules associated with the pathway determine the trajectories of chemically distinct VNO axons as they enter the AOB. These putative axon guidance cues might exhibit attractive forces, for example, guiding $CC1^+$ axons to the rostral AOB: or may have repulsive properties, inhibiting $CC1^+$ axons from growing into the caudal AOB. Recent immunohistochemical studies indicate that both types of activities may operate in the developing AOB. Most $CC1^+$ axons in the more caudal portion of the vomeronasal nerve stop abruptly, rather than continue into the caudal AOB. A small number of $CC1^+$ axons enter the caudal AOB but alter their direction by turning sharply and entering the rostral AOB. Tissue culture studies are underway to determine whether subsets of VNO neurons prefer to grow axons on substrates made from rostral vs. caudal AOB homogenates, and to examine the possibility that soluble chemoattractants play a role in directed growth of vomeronasal axons.

LHRH Neurons Migrate Along an S100-Positive Glial Substrate. D.M. CUMMINGS & P. C. BRUNJES (University of Virginia).

Luteinizing hormone-releasing hormone (LHRH) containing neurons arise in the region of the medial olfactory placode and migrate along branches of the terminal nerve to their destinations in the basal forebrain. Factors that guide LHRH neurons are beginning to be elucidated. For example, neural cell adhesion molecule (NCAM) is expressed by terminal nerve fascicles and appears to be a guidance molecule for migrating LHRH cells (Murakami et al., *Neurosci. Res.*, **12**, 1991; Schwanzel-Fukuda et al., *J. Comp. Neurol.*, **321**, 1992). Another candidate is the glial-associated protein, S100. We investigated the distribution of S100 in the developing olfactory system and forebrain of *Monodelphis domestica* (the grey, short-tailed opossum). *Monodelphis* is an ideal species for studying early development since offspring are born in a very immature state after only 14 days of gestation. Shnayder et al. (*Chem. Senses*, **18**, 1993) recently showed that S100 is expressed by ensheathing cells in the nerve and around glomeruli of both the main and accessory olfactory bulbs in developing *Monodelphis*. We also observed S100-positive cells in the olfactory nerve layer. In addition we saw S100 immunoreactivity in cells of the terminal nerve ganglion and its centrally and peripherally projecting fibers beginning at the earliest age examined, the day of birth (P0), and also on P5, P10 and P20. Double-labelling experiments further revealed that S100-immunoreactivity is co-localized with migrating LHRH neurons on P0, P5 and P10. Since S100 exerts neurotrophic effects on various cells of the embryonic central nervous system (Selinfreund et al., *PNAS*, **88**, 1991; Kligman and Marshak, *PNAS*, **82**, 1985), it may play a similar role in guiding LHRH neurons from the olfactory placode to the basal forebrain.

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Sexual and Seasonal Differences in the Vomeronasal and Terminal Nerve Systems of Terrestrial Salamanders.
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We investigated the variation in vomeronasal organ size and GnRH-containing neurons in a common terrestrial salamander, Plethodon cinereus. Salamanders in this genus have well developed main olfactory and vomeronasal organs and chemoreception has been shown to be associated with a number of behaviors, including mate recognition and courtship. Little is known about the distribution and abundance of GnRH-containing neurons in these salamanders, although these neurons have been shown to be associated with olfactory and vomeronasal nerves in distantly related salamanders (tiger salamander and rough-skinned newt). Male and female P. cinereus were collected throughout the year and immediately sectioned for vomeronasal organ volume calculations (using image analysis) and GnRH immunocytochemistry. We also examined gonads to determine stage of gametogenesis and maturation. Vomeronasal organ volume data were compared using multiple regression. Total body size and sex significantly affect vomeronasal organ volume; as body size increases, so does vomeronasal organ volume, and males have significantly larger vomeronasal organs than females at all times of the year. Vomeronasal organs are larger during the summer, prebreeding season than during any other season; summer is also the season of rapid gametogenesis. Thus, vomeronasal organs can be added to the small list of neural structures that can vary seasonally during adult life; this variation is probably linked to the neurogenesis of vomeronasal receptors throughout life. GnRH-ir neurons appear to be more heavily labelled in salamanders captured in aggregations during the breeding season than at other times of the year. GnRH-ir fibers follow olfactory and vomeronasal nerves to the telencephalon as in other salamander species.

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Male Monodelphis domestica responses to estrus-induced female odors - A bioassay for reproductive condition.
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A female S. American gray opossum (Monodelphis domestica) was induced into estrus by daily exposure to a priming male's odors by switching their nest boxes. A control female's own nest box was switched daily with another of her own boxes. The differential nuzzling (a vomeronasal investigatory behavior; Poran et al., Physiol. Behav. 53, 959-967. 1993) and scent marking responses of 10 males to the simultaneous presentation of the two female odor deposits was evaluated on alternate days for two weeks. On day 0, no preference for either female was detected. A progressive increase in the number of males preferring to nuzzle the experimental female odor was observed up to day 8, and the most significant increase in mean nuzzling duration ocurred on day 6 ($p<0.01$). This coincided with the initiation of flank and hip marking by the experimental female during nest box exchange indicative of proestrus. Male scent marking over the experimental female odor deposits significantly increased on day 4 ($p<0.01$). After day 8 an abrupt shift occurred and all males demonstrated preference for the control female odors and a highly significant drop ($p<0.0001$) in nuzzling of the experimental female odors. In conclusion, the progression into and out of estrus can be readily inferred from the pattern of nuzzling and marking responses of non-priming males. We propose this non-invasive bioassay as an alternative to vaginal smears and laparotomy for assessment of reproductive state in M. domestica.

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Male chemical cues are processed differently after uterine activation in female prairie voles.
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Chemical signals from male voles (*Microtus ochrogaster*), processed by the vomeronasal system, induce uterine growth in adult female prairie voles. The initial response to male vole urine includes neuronal activity in vomeronasal pathways as indicated by fos-immunoreactivity (fos-ir; fos is the protein product of the immediate early gene, c-fos, and is used as a marker for neuronal activity). Our results suggest an interaction between uterine development and exposure to male chemical cues on fos-ir in the premammillary nucleus. Voles with large uteri (after repeated exposures to male urine) have many fos-ir cells in the premammillary nucleus if exposed to male urine one hour before sacrifice. If similarly treated voles are exposed to water one hour before sacrifice they do not have fos-ir cells in this area. Voles with small uteri do not have fos-ir cells in the premammillary nucleus following exposure to male vole urine or water one hour before sacrifice. Thus, it appears that something that accompanies frequent exposure to male urine and/or uterine growth primes the premammillary nucleus to respond to subsequent exposure to male cues. This modifying condition may be the concentration of circulating estrogen.

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Quinine Discrimination in Rhesus Monkeys.
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An operant conditioning procedure was used to establish a quinine-water discrimination in three, food-restricted, rhesus monkeys. In a trials procedure, a visual signal indicated that lip contacts on a touch sensitive response device would result in 0.5 cc fluid delivery (quinine sulfate or water). Delivery of fluid was followed by a different set of visual signals which indicated that responses on one of two levers would result in delivery of a 300 mg food pellet. If the fluid presented was quinine, responses on the left lever led to a food reinforcer. If the fluid was water, responses on the right lever produced reinforcement. A response on the inappropriate lever led to a sixty second time-out during which responding had no effect. Each experimental session consisted 100 randomized trials: fifty trials of quinine, and fifty trials of water. The criterion for successful discriminative performance was five consecutive sessions of responding at greater than 90% accuracy; this was achieved in each of the monkeys. After the discrimination was established, test trials were conducted in which varying concentrations of quinine (*i.e.*, three, 0.5 log-unit-step reductions from the training concentration) were presented during 60 of the trials, and the training concentration of quinine or water was presented on the remaining 40 trials. Responding on the quinine lever was dependent on the concentration of the test solution. Subsequently, the original training concentration was reduced by half-log units until discriminative performance could no longer be maintained reliably (0.1 mg/ml). As the training concentration was lowered, quinine-appropriate responding was shifted to smaller concentrations. Variations of this procedure may be useful for investigations of a variety of phenomena related to taste mechanisms.

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A Novel Psychophysical Procedure for the Assessment of Bitter Taste Sensitivity in Rats. CARRIE E. PIERCE, CHRISTEN A CARSON, ROBERT J. CONTRERAS (Florida State University, Department of Psychology, Tallahassee, Fl., 32306-1051)

A persistent problem with attempts to examine bitter taste mechanisms has been the lack of adequate behavioral methodology providing data which parallels that obtained from physiological investigations. We developed a brief contact procedure whereby we measured rats licking responses to sucrose and to mixtures of sucrose with quinine. Six male Fischer 344 rats were trained to drink immediately to brief 30-s presentations of 0.3 M sucrose solution. In the first phase of testing, the animals received two random 30-s presentations each of 0.05, 0.2, and 0.8 M sucrose. In the second phase of testing, the animals received two random presentations each of water, 0.8 M sucrose solution, and 0.8 M sucrose mixed with either 0.2, 1.0, or 5.0 mM quinine hydrochloride. A microcomputer controlled stimulus presentations and measured the animal's licks of each solution during each 30-s presentation. In Phase I, the number of licks increased monotonically with sucrose concentration, with means of 15.3, 75.8, and 156.9 licks/30 s for 0.05, 0.2, and 0.8 M sucrose, respectively. In Phase 2, the rats averaged 148.7 licks of sucrose and 12.7 licks of water. The number of licks decreased monotonically with quinine concentration, with means of 64.2, 20.3, and 6.3 licks/30 s of 0.8 M sucrose mixed with 0.2, 1.0, and 5.0 mM quinine, respectively. These results demonstrate rats' acute ability to discriminate by taste not only the presence but the concentration of a dilute bitter compound dissolved in a strong sucrose solution. We will determine the lick rate functions to mixtures of sucrose with other bitter compounds of the same concentration. This will provide a means to determine functional similarity and dissimilarity among various bitter compounds. These results can then be compared to equivalent data obtained from neural studies.

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Short-Term Taste Tests of Sucrose and Saccharin in the Laboratory Rat

JODI RHINEHART-DOTY (Florida State University) and JAMES C. SMITH (Florida State University)

Smith et al. have shown that the number of licks a rat makes in a 30 second period is a direct function of concentration for a variety of carbohydrate solutions. Since the previously published data were collected using sucrose, glucose, fructose and maltose in very short-term tests, it is likely the functions are the result of the taste of the compounds and little, if any, from post ingestional feedback. The short-term apparatus used in the Smith et al. and present study consisted of a small testing cage where any one of eight different drinking tubes could be presented to the rat when a shutter was opened. A contact type lickometer allowed for measurement of inter-lick-intervals with a resolution of 1 ms. Once the rat took the first lick, that concentration of the compound was available for 30 seconds before the shutter was closed, not allowing further access to that particular tube. Another concentration of the solution was then presented to the rat again allowing a 30 second access period after the first lick. In the work presented here, the response to six concentrations of sucrose (0.03 to 1.0M) in these short-term tests are compared to seven concentrations of sodium saccharin (0.001 to 0.066M). The sucrose solutions yielded an increasing monotonic function with increases in concentration. The saccharin concentrations yielded an inverted U function, which peaked at .004 M and then decreases with higher concentrations. One can infer that the best tasting saccharin to the rat is .004 M and as the concentration increases the rat consumes less, presumably because of the bitter taste. These short-term data are compared with long-term two bottle preference data and a rationale is given for the efficacy of the short term tests for taste measurements.

Preference for L-Amino Acids in Rats With or Without L-Lysine Deficiency: The Effect of Bilateral Taste Nerve Neurotomy.

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L-Amino acids (AA) in plasma and brain of male rats remain unchanged when a normal diet is available. When an L-lysine (Lys) deficient diet is offered to rats, Lys in plasma and brain declines. When solutions of various individual AAs were offered to the Lys-deficient (Lys-def) rats, they selected the Lys solution, and their food intake and growth normalized. The recording of single neuron activity in the lateral hypothalamic area (LHA) of these rats suggested that neural plasticity occurred, with some neurons specifically responding to the deficient nutrient. This neural plasticity in the LHA was highly responsive to the specific AA ingested under protein or Lys deficiency and was long-lasting, even after the control diet became available. Gustatory information during ingestion of AA solutions is the most important cue for the maintenance of AA homeostasis. Taste preferences in rats with bilateral neurotomy of the chorda tympani (CTx) and/or the glossopharyngeal (GPx) nerves were studied when rats were fed a normal or without Lys-def diet. Preferences were measured in a choice paradigm where rats were able to choose among several AAs. In this condition, intact rats chose Lys when they were Lys-def and selected monosodium glutamate (MSG) and L-arginine (Arg) when they were on a replete diet. In contrast, CTx rats exhibited no change in preference for Lys or Arg, regardless of their nutritional state (Lys-def or replete). In contrast, their preference for MSG declined in the Lys-def state. The preferences of GPx rats were essentially the same as those of intact ones in both nutritional conditions. Preference for AAs in rats with both CTx and GPx appeared comparable to that seen in rats with CTx alone for most AAs tested. Additionally, both the most preferable concentration and the total intake of Lys in normal rats were shifted to a significantly higher level when fed a Lys-def diet. These data suggest that the CT nerve plays an important role in control of AA preference in rats fed both a normal and Lys-def diet.

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The Effects of Gustatory Nerve Section on Concentration-Dependent Licking to Maltose in Rats. RACHEL REDMAN, MIRCEA GARCEA, and ALAN C. SPECTOR (Dept. of Psychology, Univ. of Florida).

Recent behavioral results in the rat suggest that the collective input of the 7th cranial nerve is necessary and probably sufficient for the normal maintenance of suprathreshold responsiveness to sucrose. This study examined whether these findings would extend to another disaccharide, maltose. Water-deprived rats were first trained to lick water from a drinking spout during two 30 min sessions in a specially designed gustometer. In the next 3 daily sessions (40 min) rats were presented with 10 s trials of water and 6 concentrations (0.01-1.0 M) of maltose in repeated randomized blocks. The rats were then similarly tested in a non-deprived state for 3 days both before and after surgery. Each rat's responsiveness to each concentration was standardized by subtracting licks to water from licks to maltose (lick difference scores). Both before and after surgery, lick difference scores monotonically increased as a function of concentration. After combined transection of the greater superficial petrosal (GSP) and the chorda tympani (CT) nerves (GSPX+CTX, n=7), rats significantly decreased their maltose responsiveness, especially to high concentrations. Similar results were obtained in rats that received combined transection of the CT and the glossopharyngeal (GL) nerves (GLX+CTX, n=8), and in rats that had their sublingual and submaxillary salivary glands extirpated (DESAL, n=10). Sham surgery (n=9), CT section (n=7), and GL section (n=7) alone were without effect. All nerve sections were histologically confirmed. When rats were water-deprived and tested for their continuous spout licking of water, the local lick rate decreased significantly after surgery in the GSPX+CTX and DESAL groups by 1.01 and 0.54 licks/s, respectively. This apparent motor effect, however, was not sufficient to account solely for the decreased maltose responsiveness observed in these groups. These results suggest that, as with sucrose, the collective gustatory input of the 7th cranial nerve, as well as the presence of the submandibular and submaxillary salivary glands, is necessary to maintain normal unconditioned responsiveness to maltose. Overall, these findings imply that there is a substantial central convergence of maltose-relevant input from the various gustatory nerve branches.

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Glossopharyngeal Nerve Transection Does Not Compromise Cation Specificity of Depletion-Induced Sodium Appetite in Rats.
STACY MARKISON, STEVEN J. ST. JOHN, and ALAN C. SPECTOR (Department of Psychology, University of Florida).

Previous findings suggest that the chorda tympani nerve (CTn) is critical in the cation specificity of depletion-induced sodium (Na+) appetite in rats. This study was conducted to determine the respective effects of glossopharyngeal nerve (GLn) section and sublingual + submaxillary salivary gland removal on taste-guided licking behavior to salts by Na^+ depleted rats. Water-deprived rats were first trained to lick water from a drinking spout in a specially designed gustometer. The rats were then presented with 10 s trials of water and 0.1 M sucrose during 2 additional sessions. Next, rats received either bilateral section of the CTn (CTX, n=6), bilateral section of the GLn (GLX, n=6), extirpation of the sublingual + submaxillary salivary glands (DSAL, n=6), or sham surgery (CON, n=7). Following recovery, rats were injected with the diuretic furosemide, and given Na^+ deficient food. During the next 24 hours rats excreted an average 2.69 mmol of Na^+, inducing a Na^+ depleted state. The Na^+ depleted rats were tested for their responsiveness to water, and 0.05 M and 0.3 M concentrations of NaCl, KCl, $CaCl_2$, and NH_4Cl during 10 s trials presented in randomized blocks of nine. Rats were subsequently retested in a water-deprived state with water and 0.1 M sucrose to assess their licking competence. Bonferroni-adjusted paired comparisons indicated that rats in the CON and GLX groups licked both Na^+ solutions significantly more than all other stimuli. In addition, licking to nonsodium salts was either less than or similar to that of water. In contrast, the Na^+ responses in the CTX group were not significantly different from water, KCl, or NH_4Cl. The response profile of DSAL rats was similar to that of the CTX group. However, the substantially lowered licking rates to water and sucrose under water-deprived conditions after surgery raises the possibility that general licking impairments may have contributed to the decreased responsiveness to Na^+ in the DSAL animals. These findings imply that the CTn, but not the GLn, is necessary for the maintenance of Na^+ specific, taste-guided behavior.

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The Effect of Thalamic Lesions on Primate Taste Preference.
STEVE REILLY and THOMAS C. PRITCHARD. (The Pennsylvania State University, Hershey, PA 17033)

Electrophysiological and neuroanatomical studies have identified the parvicellular division of the ventroposteromedial nucleus of the thalamus (VPMpc) as the thalamic gustatory relay. Little, however, is known about the role of VPMpc in taste perception. The only 2 neurobehavioral studies conducted with monkeys were designed to confirm the location of the thalamic taste relay rather than identify its functional significance (Blum et al., 1943; Patton et al., 1944). The present report describes 2 experiments that assessed the impact of bilateral thalamic lesions on the taste preference Old World monkeys (*M. fascicularis*). Experiment 1 used a 30 min single bottle preference test (Pritchard et al., 1994) and a battery of 12 taste stimuli to test the lesioned monkeys (n=5) and an unoperated control. The electrolytic lesions were placed in VPMpc under electrophysiological guidance. On the basis of histology, the monkeys were partitioned into 2 groups. Group GTX2 consisted of 2 monkeys with large, bilaterally symmetrical lesions that completely destroyed the posterior part of VPMpc. Group GTX3 consisted of 3 monkeys whose asymmetrically placed lesions destroyed the posterior VPMpc on one side, but spared part of the contralateral nucleus. During post-lesion testing both groups decreased their consumption of 0.003M quinine hydrochloride (QHCl; $p < 0.04$) and increased their consumption of 0.1M sodium chloride (NaCl; $p < 0.04$). GTX2 monkeys also showed an attenuated intake of 0.01M, 0.1M, 0.3M, and 1.0M glucose ($p < 0.05$). In contrast, GTX3 monkeys showed significantly elevated post-lesion consumption of 1.0M glucose, and 0.03M and 0.1M polycose. Intake of distilled water remained normal in all monkeys. In Experiment 2, there was no convincing evidence of lesion-induced deficits for QHCl, NaCl, or glucose in a 24-hr two-bottle preference test. These data, which contradict the studies cited above, indicate that VPMpc lesions in Old World monkeys produce a constellation of subtle taste impairments rather than a global ageusia or hypogeusia.

Supported by DC-00246

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The Effects of Methimazole on Olfactory Function. **LLOYD HASTINGS, STACEY ANDRINGA, and MARIAN L. MILLER (University of Cincinnati)**

It has recently been reported that methimazole (METH), a compound used in the treatment of hyperthyroidism, is a potent olfactotoxin (Genter *et al.*, 1993). Exposure to METH (300 mg/kg, i.p.) produced complete destruction of the olfactory epithelium while lower doses produced correspondingly less damage. Given the fact that morphological or biochemical insult to the olfactory epithelium does not always correlate with functional deficits, we investigated the effects on METH on olfactory function. Rats were trained on both odor and visual discrimination tasks and then injected with METH (300 mg/kg, i.p.). Anosmia was observed two to four days after exposure and lasted for approximately 30 days. Performance of the visual discrimination task was not affected, or only slightly so in a few cases, suggesting that the deficits on the olfactory task were not due to alterations in motivation or cognitive performance. An interesting observation was that when recovery occurred, it proceeded quite rapidly, *i.e.*, within a matter of a couple of days. Morphological examination of the olfactory epithelium revealed that METH caused a total sloughing of the septal olfactory epithelium, down to the lamina propria, within 48 hours of exposure. METH also caused serious depletion of secretory droplets, leaving the appearance of vacuolization of the Bowman's glands. Given the extensive morphological damage and the fact that METH is metabolized exclusively by the flavin-containing monooxygenase system present in both mucosa and Bowman's glands, it appears that the loss of olfactory function--at this exposure level--is due to a toxic action and not to alterations in hormonal status.

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Conditioned Preferences for Food Odors in Weaning Infants: Preliminary Results. TED MELCER (San Diego State University), LISA CAPO (Murray State University)

The transition from nutritional dependence on mother's milk to recognition and preferential ingestion of solid food has obvious importance for growth in normal mammalian development. An extensive literature in various animal species indicates that associative learning plays an adaptive role in acquisition of initial food preferences but surprisingly little work exists using a similar approach to studying the mechanisms controlling initiation of solid food intake in human infants at weaning age (4-months-old). The present experiment tested the possibility that infants might acquire preferences for food odors (almond/peppermint) through their association with a sweet flavor (apple juice). The conditioning trials consisted of a brief odor exposure following by a drop of fluid on the tongue. On S+ trials, one odor was always followed by a drop of apple juice and on S- trials a second odor was always followed by a drop of water. The odor (almond or peppermint) paired with the sweet flavor was counterbalanced across subjects. Infants were videotaped and the number of mouth opening responses during the S+ and S- odor presentations were scored. The results showed a significant increase in mouth openings during both S+ and S- odors across the first 12 trials. Interestingly, during the second 12 trials infants showed a trend for more mouth openings during the S+ odor than during the S- odor. These results suggest that an ingestive behavior such as mouth openings can be modified through relatively limited experience and that infants may be able to rapidly form positive associations between an odor and a sweet flavor. This conditioning procedure may be useful for investigating conditions that promote formation of preferences for food odors by infants at weaning age.

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The Human Infants' Responses to Flavored Milk. Julie A. Mennella, Carol Staley, Monica Firely and Gary K. Beauchamp (Monell Chemical Senses Center, Philadelphia, PA)

Like the milk of other mammals, human milk varies in flavor because selected volatiles from the mother's diet are transmitted to her milk. For example, we previously demonstrated that the flavor of human milk was altered when nursing women ate garlic and their infants breast fed longer when the milk was flavored with garlic than when this flavor was absent. The present studies expanded upon these findings and focused on the human infants' responses to the flavor of vanilla during breast or bottle feeding. In the first study, we monitored the sensory changes in their milk and the behavior of their exclusively breast-fed infants when lactating women consumed vanilla in a propylene-glycol base and when they consumed the diluent alone. The data show that vanilla ingestion significantly and consistently increased the perceived intensity of the milk odor and the infants spent significantly more time attached to the nipple when their mothers ingested the vanilla flavor compared to when their mothers consumed the diluent. In the second study, we monitored the behavior of formula-fed infants when they were feeding infant formula flavored with either propylene-glycol based vanilla or the diluent alone. A variety of methods were used to assess their responses and the data are forthcoming.

This research was supported in part by NIH Grants AA09523 and DC00882. Dr. Mennella is a Morley R. Kare Fellow.

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Temporal Changes in Feeding Patterns Following Peripheral Injections of Bombesin-Like Peptides

A. KURT THAW (Florida State University), JAMES C. SMITH (Florida State University) and JAMES GIBBS (Cornell University Medical College)

Peripheral injections of bombesin-like peptides have been shown to extend the interval between meals in ad libitum feeding Sprague-Dawley rats in this laboratory. The present study investigated how such changes in feeding behavior may effect the normal (baseline) pattern of consumption over a 24 hour period. Eight subjects were allowed ad libitum access to powdered food and water for the entire study. Following 10 days of baseline data subjects received a peripheral injection of either saline or a bombesin-like peptide following the first night meal. Injections continued to be administered until all peptides had been tested twice. Subjects received only one injection per day and there was a minimum of 48 hours between any two peptide injections. The peptides tested include: bombesin (4ug/kg and 8ug/kg), gastrin releasing peptide 27 (8ug/kg and 16ug/kg), gastrin releasing peptide 10 (8ug/kg and 16ug/kg) and neuromedin B (8ug/kg and 16ug/kg). A total of 10 saline injection days were included as control data. Results indicate a monotonically increasing feeding curve following saline injections, but not bombesin-like peptide injections. However, both lines intersect at around hour eight of the dark phase.

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The Taste of Sucrose Reduces the Salt Intake of Sodium Deficient Rats Even After a Delay of Six Hours. SANDRA P. FRANKMANN (University of Southern Colorado) and JOHN H. DOKKO (Cornell University Medical College).

Sodium depletion produces a salt appetite and increases the acceptability of normally rejected NaCl solutions. In previous work, we have demonstrated that 1) sucrose can substitute in part for NaCl in the satiation of salt appetite and 2) the effect of the sucrose is due to oral (taste) and not postingestive (i.e. osmotic, etc.) stimulation. In these studies, the intake of NaCl solution was reduced by 64% when access to the NaCl solution was immediately following intake of the sucrose solution. The immediate contrast between the highly palatable sucrose solution may have influenced the perceived palatability of the NaCl solution thus accounting for the suppression. If this were true, then imposing a delay between the sucrose solution and the NaCl solution intakes might result in an attenuation of the suppressive effect of sucrose on the salt appetite. To test this, male rats were sodium depleted (10 mg furosemide and overnight sodium deficient diet) and their salt appetites tested (1 h access to 0.3M NaCl and water) following no sucrose or 15 min access to 0.6M sucrose. The sucrose was offered either immediately prior to or 6 h prior to the salt appetite test. Each rat was tested under all four conditions: 1) no sucrose, no delay; 2) no sucrose, 6h delay; 3) sucrose, no delay and 4) sucrose, 6 h delay. The two no sucrose conditions resulted in equivalent 1h intakes of 0.3 M NaCl (12.5 ± 0.8 ml (no delay); 13.2 ± 1.2 ml (6 h delay)). The 15 min sucrose intakes were not significantly different for the two delay conditions. There were significant decreases of 0.3M NaCl intake whether the sucrose was ingested immediately prior to the salt appetite test (46%) or 6 h prior to the salt appetite test (52%). There was no significant difference between the intake of 0.3M NaCl following sucrose intake immediately prior to or 6 h prior to the salt appetite test. Thus, the suppressive effect of sucrose on NaCl solution intake persists for at least 6 h and does not diminish in potency over this time interval. The suppression of salt appetite by sucrose does not depend on an immediate contrast effect between the taste of sucrose and the taste of NaCl.

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Microstructural Analysis of the Effects of Sucrose Concentration and Food Deprivation on Licking Behavior in the Rat. PERRIN A. KLUMPP and ALAN C. SPECTOR (Dept. of Psychology, University of Florida).

Research of drinking behavior in the rat has been dominated by measures of fluid volume, preference, and avoidance. Rather than quantifying the outcome of behavior, microstructural analysis of licking focuses on the temporal organization of the behavior itself, thus providing a basis for the study of the neural and physiological substrates of ingestion. Six rats were trained to lick a sucrose solution through a drinking spout. The onset time of each lick was recorded by a computer. All subjects were tested on an ascending series of concentrations (0.03M, 0.1M, 0.3M and 1.0M). Each concentration was presented for 1 h on 6 consecutive days; in 3 of these sessions the rats were 23 h food-deprived. Drinking is temporally organized into periods of sustained licking (bursts) separated by pauses of varying duration. We selected various durations (300 ms - 300 s) to be used in defining when an interruption in licking was sufficient to be considered a pause, constituting the end of a burst. For all concentrations, food deprived subjects consumed a significantly greater volume of sucrose. Intake increased as a function of sucrose concentration up to 0.3 M, and then tended to decrease at 1.0 M. Using a liberal pause criterion such as interruptions in licking equal to 300 ms or greater to define a burst of licking (a commonly employed definition), these changes in total intake were primarily accounted for by proportional changes in the number of bursts; burst duration was generally unaffected by concentration or deprivation state. As the pause criterion became progressively longer, however, burst duration increased as a function of concentration. In addition, the curves describing the number of bursts as a function of concentration and deprivation state substantially changed. Regardless of pause criterion, the within-burst lick rate was relatively unaffected by either concentration or deprivation state. Contrary to expectations, there was no strong or systematic correlation between burst duration and the subsequent or preceding pause, irrespective of pause criterion. In addition to demonstrating that concentration and deprivation state affect various drinking-pattern parameters in different ways, these results stress the importance of including a wide range of criteria for defining pauses, and thus bursts, in an microstructural analysis of ingestive behavior.

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Durability of conditioned taste preferences
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J. JAY BRAUN (Arizona State University)

Preferences for taste stimuli associated with calories during training were tested 1 day-4 wks later under 24 hrs food deprivation. During 4 consecutive training days, 18 Long Evans hooded rats had simultaneous access to 2 graduated cylinders containing 3.42% sucrose (calories) and 0.012% saccharin (no calories). For 9 rats, 0.2% NaCl was dissolved with the sucrose and 0.031% citric acid with the saccharin; the other 9 had the reverse condition: 0.031% citric acid cueing sucrose and 0.2% NaCl cueing saccharin. Bottle positions were reversed during daily fluid intake measurements. For the 6-hr, 2-bottle tests, the same NaCl and citric acid concentrations were either dissolved in water for preference choice; or, the 2 stimuli were presented against a background of a low caloric sweet mixture (1.71% sucrose + 0.006% saccharin, w/v) in each cylinder. During training the sucrose mixture was highly preferred (~96%). Testing results indicated that tastes (NaCl or citric acid) previously paired with calories were preferred 1 day after training when the background was water in both cylinders (around 65% preference) or when the 2 stimuli were dissolved in the sweet mixture (around 78% preference), supporting prior conditioned taste preference results (Holder, M.D., Appetite, 17: 29-45, 1991). Preferences for the calorie cue stimulus were maintained over nontraining gaps of 1, 2 and 3 wks when dissolved in water (61%, 72%, 56%, respectively) or in sweet mixture (70%, 83%, 76% after 2, 3 and 4 wks, respectively). Overtraining had a minimal impact. It is concluded that conditioned taste preferences are retained for at least a month.

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Taste Aversion Conditioned with Exposure to a High Energy Magnet
JAMES C. SMITH, ROSS P. HENDERSON, JODI RHINEHART-DOTY & LISA T. PEGUES (Florida State University)

Magnetic resonance imaging procedures using 1-2 Tesla magnets are widely used and considered to be safe. There is considerable pressure to produce and use much more powerful magnets in order to increase resolution, especially in brain imaging diagnostics. The general biological and the neurological hazards of exposure to 4 Tesla (and above) magnets are unknown and worrisome (See Fitzgerald, K. 1993 Magnetic apprehensions. *Sci. Am.* 269:106-107). In past research, it has been shown that one of the most sensitive biological assays of ionizing irradiation was the use of "taste aversion learning". By analogy, we utilized this procedure to ascertain if taste aversions could be conditioned using intense magnet exposure as the unconditioned stimulus. For the experiments, male laboratory rats were accustomed to taking their daily water intake during a 20 min. period early in the light cycle. On the conditioning day a novel saccharin solution was substituted for the water and the rats were subsequently exposed to a 9.4 Tesla permanent magnet for 15 minutes. For the exposure, the rats were confined in a Plexiglas chamber and elevated through the fringe field to the magnet core. Sham exposed animals were likewise confined in another similar tube for the 15 min. period. Rats exposed to the magnet showed a profound aversion to the saccharin which lasted for approximately 10 days. Additional experiments will be reported that were conducted to gain information about the nature of the unconditioned response.

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The paradox of discriminatory nondiscriminators revisited
MICHAEL O'MAHONY, JEANNINE DELWICHE, RIE ISHII, and SUSUMU MASUOKA (Dept. of Food Science & Technology, University of California, Davis)

For triadic difference testing, a paradox was noted whereby a slight alteration in the instructions for a triangle test to those of a 3-AFC, resulted is discrimination performance improving. This surprising result has been explained in terms of Thurstonian modeling by a change in cognitive strategy. Essentially, the judge uses a 'skimming' rather than a 'comparison of distances' strategy in the 3-AFC, which results in superior performance even though d' remains the same. The two strategies were considered for tetrad testing. The predictions were not the same; slightly worse performance was predicted for 'skimming'. Predictions were tested and confirmed using as target stimuli, a base chocolate pudding and a slightly sweetened pudding, with other flavors as disractors for strategy maintenance.

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Creaminess Perception
E.A. SKIBBA (University of Missouri-Columbia)
H. HEYMANN (University of Missouri-Columbia)

Little is known about the factors which influence creaminess perception. The primary purpose of this research was to define the concept of creaminess in food products. Twenty food products were evaluated for mouthfeel and texture attributes using free-choice profiling (FCP) methodology. Further, multidimensional scaling (MDS) was used to classify the twenty products by creaminess similarity. The FCP panel generated between six and ten terms to describe the mouthfeel/texture of the products. The three-dimensional consensus space derived from the Procrustes analysis explained 77.7% of the variation among the samples. Samples were classified by thickness, smoothness and stickiness. In the MDS, subjects formed between three and ten products categories. The two-dimensional MDS solution explained 94% of the variance. Samples appeared to be divided by thickness and homogeneity. The FCP and MDS panels similarly classified the food samples along two dimensions. This study showed that creaminess is explained by the perception of oth smoothness and thickness.

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Ultrathin Flexible Endscopy of the Human Olfactory Cleft

DONALD LEOPOLD(Johns Hopkins Medical Institutions)

Direct examination of the human olfactory cleft offers the possibility of obtaining information which will be helpful in the clinical management of patients with olfactory complaints. This examination has been difficult because of the narrowness of the cleft and its location behind other nasal structures. Techniques utilized usually have included the application of a topical anesthetic followed by dilation of the cleft with a speculum and/or the insertion of a rigid endoscope. These techniques can be uncomfortable, cannot be utilized in every patient, are time consuming, and produce pharmacologic and traumatic changes to the mucosa in the olfactory cleft. Using a newly available, .8 mm flexible fiberoptic instrument (Xomed Trease, Inc., Jacksonville, Fla), endoscopy of the olfactory cleft is now possible in all patients without the use of any topical nasal preparation or dilation. With this endoscope, the olfactory cleft in humans has been noted to be lined by variably thick epithelium which is a pale pink color and is difficult to distinguish from respiratory epithelium. At this early stage of its use, there has been no observable correlation between physical findings and clinical olfactory status. Its use however can sometimes eliminate the need for CT scan or MRI imaging to determine the patency of the olfactory cleft. Inflammation in the cleft can also be relatively easily diagnosed. With refinements in these techniques, it should be possible to utilize this endoscopy in all patients with olfactory complaints.

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A New Technique of Multichannel Magentoencephalographical Recordings of Cortical Responses to Chemical Stimulation in Man

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After chemical stimulation of the human nasal mucosa it is possible to record multichannel chemosensory event-related potentials (CSERPs). Despite the possibility of mapping these responses localization of the underlying neuronal generators is difficult. Recently magnetoencephalographical methods have been developed to record chemosensory event-related magnetic fields (CSERMFs). Although both CSERP and CSERMF are epiphenomena of transmembrane currents it has been proposed that CSERMFs are almost entirely due to membrane currents rather than to the extracellular volume currents that are responsible for the electrical responses. Also, the CSERMFs are not modified by the physical properties of the conductive medium resulting in a more reliable source localization.
Twelve healthy volunteers (5 male and 7 female subjects; 20 to 40 years) participated in the experiments. Stimuli (70% v/v CO_2) were presented with a duration of 200 ms and an interstimulus interval of 20 s. The magnetoencephalogram (MEG) was recorded with a 37 channel superconducting first order axial gradiometer (KRENIKON) in a magnetically shielded chamber. Additionally, an magnetic resonance image scan (MRI) was performed of each subject in order to transform biomagnetic coordinates into MRI coordinates. Also, one EEG channel was recorded from Cz versus A1 according to the international 10/20 system. Eye blinks were monitored via the Fp2 lead (referenced against A1).
It was possible, in all subjects to obtain CSERMFs and to localize the underlying neuronal sources. Presentation of the trigeminal stimulant CO_2 resulted in a source localization in the vicinity of the central sulcus. When presenting the odorants vanillin and hydrogen sulfide in a similar experiment the data indicated that the trigeminal source can clearly be separated from the activity of other non-somatosensory projection areas such as the primary olfactory cortex.

This research was supported by DFG grant Ko 812/5-1

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EEG Responses to Odors Vary With Cognitive State and Prior Experience. W. R. KLEMM, S. D. LUTES, D. V. HENDRIX (Chemical Senses Laboratory, Texas A&M University), and S. WARRENBURG (International Flavors & Fragrances Co., Union Beach, N.J.)

We tested two hypotheses about the brain's responsiveness to continued exposure to odors by recording the spontaneous EEG from college-age females. One hypothesis was that EEG changes would vary, depending on the cognitive state of subjects during odor presentation. The second hypothesis was that frequency- and topographic-specific EEG changes would occur as a given odor trial was repeated.

Eighteen subjects were tested with a counterbalanced sequence of five odors (blank, baby powder, birch tar, lavender, lemon), repeated five times. Each odor-presentation trial included a 20-sec no-odor baseline, followed by 20 secs of odor delivery (2L/min, via face mask). The same pattern of testing was conducted under two cognitive conditions, vigilant (subjects had to identify the odor and signal when it first came on) and emotional (subjects gave affect scores for each odor on an aoursal-sleepiness continuum and a pleasure-displeasure scale).The EEG from 19 electrodes was evaluated by frequency spectral analysis.

Cognitive state did seem to have an effect. When vigilance and emotional data were considered separately, theta activity showed a significant odor x region effect during vigilance (P=0.02, MANOVA). Increases in theta over posterior regions on the right side were most evident in response to lemon. Much different odor patterns occurred during emotional trials, but lemon showed great increases over all regions.When vigilance and emotional data were considered as variables in the same analysis, collapsed over replicates, univariate ANOVA revealed a significant cognition x region effect (P = 0.005) in the alpha$_1$ band, due to increases in most regions during vigilance. At the same time, alpha$_2$ activity increased in all regions during the emotional trials (P = 0.026).

Replication effects were also evident. During emotional trials, activity in the alpha$_1$ band revealed a complex odor x replication effect (P = 0.008, MANOVA). A significant replication effect (P = 0.05, univariate ANOVA) was noted in the alpha$_2$ band in both the vigilant and emotional conditions. The amount of alpha$_2$ activity decreased in the third replicate, but then rebounded to near-initial levels by the last trial.

These results seem to confirm that both cognitive states and prior experience do influence the brain-wave response to odors.

This research was supported in part by International Flavors & Frangrances, Inc.

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Volumetric Analysis of Nasal Airways via Artificially Intelligent Computer Analysis of MR Images MICHAEL FONTE, NIKOLAUS SZEVERENYI, DAVID E. HORNUNG, and DANIEL B. KURTZ (SUNY, Health Science Center, Syracuse, N.Y. 13210)

A volumetric analysis of Computer-assisted Tomography (CT) images has demonstrated a relationship between olfactory ability and nasal anatomy. Although the radiation exposure associated with a CT is minimal, the expansion of this research to subjects necessitated the development of a technique employing magnetic resonance imaging (MR). Fast spin echo, spin density MR images were found to provide the best signal to noise ratio, allowing for the most accurate delineation of the nasal airways. To reduce bias in the determination of nasal compartment geometry, an artificially intelligent (AI) computer program was developed. Nasal airways were divided into 32 regions or compartments by the AI program. Rather than simply projecting a grid over the images to characterize nasal geometry, individual dividing lines were computed based on readily identifiable landmarks (e.g. cribriform plate and hard palate). Data from 5 sagittal scout images and 30 coronal sections were analyzed on a SUN workstation using a mix of commercial (IMAGE, NMRi) software and custom-written programs. Since MR images are digital in nature, complex volumetric data can be quickly and reproducibly extracted with these computer techniques. These compartment volumes can now be combined with measures of olfactory ability to allow for a correlation between nasal anatomical variations and olfactory dysfunctions in both patient and subject populations.

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Ambient Odor: Effect on Consumer Decision-Making.

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Two studies explored the role of the congruency of pleasant odors on consumer decision-making in a laboratory setting. In both studies there were three odor conditions (no experimental odor, chocolate scent or floral scent) which were crossed with two product classes (floral arrangements or chocolate assortments). In the first study, subjects were given a computer scenario where they had to choose which of four flower arrangements (or chocolate assortments) they liked best, based on information they acquired concerning six attributes. They could examine as much of the attribute information as they desired. Measures included the pattern and amount of information-search conducted. In the second study, subjects were given a computerized scenario in which they had to choose from a list of seven flower arrangements (or chocolate assortments) 21 times. Measures included several variety-seeking behaviors. In the first study, subjects in the congruent odor condition were more holistic in their processing (looking more evenly at all the attributes) and were more likely to choose the least preferred option (thus spreading their choices more evenly over the four alternatives) than subjects in the incongruent odor condition. In the second study, subjects in the congruent odor condition switched more among the seven choices, chose items that were rated more dissimilar, and chose the least favorite item more frequently than did subjects in the incongruent odor condition.

This research was supported in part by a grant from the Olfactory Research Fund.

Monitoring Activity in Human Olfactory Epithelium: Odorant Induced Changes in Reflected Light

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Evaluation of olfactory dysfunction at the site of initial transduction is currently only by inference from olfactory biopsies or through direct visualization with endoscopy. A technique that could monitor the initial olfactory transduction events across a large portion of the mucosa may shed light on both basic mechanisms of olfactory function and dysfunction. Optical techniques coupled with video technology offers such a possibility. Using the excised olfactory epithelium of the rat as an *in vitro* preparation, we have recorded odorant induced optical changes with both intrinsic reflection signals and an extrinsic florescent probe Di4Anepps. Although the signal was larger for the extrinsic probe, the intrinsic signal was surprisingly large, sometimes measuring 0.2%. The spatial patterning of odorant responses across the olfactory mucosa was nearly identical for both extrinsic and intrinsic signals, suggesting they monitor the same olfactory events.

Extrinsic signals (those recorded using fluorescent dyes) work well with animal experiments, but these dye probes are not approved for use in humans due to possible pharmacological interactions and phototoxicity. Optical imaging of intrinsic signals is without these potential risks. Therefore, we developed a high resolution optical functional map of *in vivo* human olfactory mucosa using an intrinsic reflection signal. We have adapted a rigid nasal endoscope to record intrinsic reflection signals with a COHU CCD camera. The excitation light (620 nm) was focused onto a 4 mm fiberoptic bundle connected to an endoscope.

Presently, using rigid endoscopy in humans, we can visualize approximately 50% of the olfactory epithelium. We have observed a 1% change in reflectivity in the human olfactory mucosa in response to amyl acetate. The response was only observed in the olfactory cleft and not on areas containing primarily respiratory epithelium. In addition, very little change was observed when the olfactory epithelium was exposed to non-odorized air. Additional controls are currently being done to understand the source of these signals. Nevertheless, we seem to be able for the first time, to evaluate olfactory function at the initial site of transduction.

Electrical Responses Obtained from the Human Olfactory Epithelium

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After chemical stimulation of the human olfactory epithelium it is possible to record negative responses (electro-olfactogram, EOG) which are interpreted as the summated receptor potentials of the olfactory nerve. The aim of this investigation was to test the EOG´s changes in relation to both stimulus duration and length of the interstimulus interval (ISI). Stimulation was performed with substances believed to eclusively excite fibers of the olfactory nerve (vanillin 2.08 ppm; hydrogen sulfide 0.78 ppm). Twelve healthy volunteers (5 male and 7 female subjects; 25 to 40 years) participated in one or two sessions performed on separate days. Duration of sessions ranged from 30-120 min. Stimulants were presented with 3 stimulus durations (250, 500, and 1000 ms; ISI 60-120 s). In addition, pairs of stimuli were applied at 4 ISIs (1024, 2048, 4096, and 8192 ms; stimulus duration 1000 ms). EOG was recorded by means of tubular electrodes (cutaneous reference contralateral bridge of the nose; impedance < 2 kΩ; bandpass DC to 70 Hz, sampling rate 125 Hz). In addition, eye blinks were monitored via the Fp2 lead (referenced against A1). EOG could be recorded in 7 out of 12 subjects. After olfactory stimulation (1024 ms) the responses' duration ranged from 2 to more than 10 s; the peak amplitude was reached within 2 s after stimulus onset. Amplitudes were found to range from 0.01-1.8 mV. In all subjects both amplitudes and areas under the curves increased in relation to an increase in stimulus duration. When pairs of olfactory stimuli were applied responses obtained at an ISI of 8192 ms were clearly separated from each other. In contrast, at an ISI ≤ 2048 ms responses were superimposed on each other. As with an ISI of 8192 ms, the amplitude produced by the second stimulus was as great as the first responses' amplitude indicating that the peripheral encoding is not subject to desensitization.

Cyclic AMP Directly Activates Chloride and Cation Conductances in Olfactory Receptor Neurons from the Mudpuppy, *Necturus maculosus*.

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Odors are transduced by selectively modulating several different conductances in mudpuppy olfactory receptor neurons (ORNs), including odor-elicited increases in Cl^- and nonselective cation (NSC) conductances. The olfactory responses fade quickly during normal whole-cell recording, consistent with a dependence on second messengers, but the specific messengers are not known. To study this question, we have used flash photolysis to release cyclic nucleotides in isolated olfactory neurons while recording the whole-cell current with patch electrodes. NPE-caged compounds were perfused into cells from patch pipets and released by brief (1 ms) UV light flashes. Photolysis of NPE-caged cAMP induced a fast, transient inward current at -90 mV in 53/66 ORNs. The current varied from cell to cell: peak = -49 ± 10 pA (n=53, range -3 to -376 pA); time-to-peak = 390 ± 30 ms (n=47); decay half-time = 1.3 ± 0.17 s (n=34). Similar responses were produced by ciliated dendrites isolated without somas (n=2) and by NPE-caged cGMP (n=7). No response was elicited by light alone (n=4) or by the NPE-cage and proton released during hydrolysis (n=10). Reversal potentials (E_{REV}) were measured in 25 cells to identify the ionic basis of the cAMP-stimulated currents. In 7 cells the response reversed at -32 ± 2.4 mV, near E_{Cl} (-35 mV), while in 4 cells the response reversed at -1 ± 1 mV, near E_{NSC} (~0 mV). But in most cells E_{REV} was near -20 mV, between E_{Cl} and E_{NSC}. Voltage ramps revealed a time-dependent change in E_{REV} during the 2 s after a flash indicating that the NSC conductance activated transiently while the Cl^- conductance activated and was sustained (n=7). The cAMP-stimulated Cl^- current was not caused by Ca^{2+} entry through NSC channels because the Cl^- current often activated first, sometimes activated alone, and could be elicited when cells were bathed in 0 Ca^{2+} saline buffered with EGTA (n=2). The data are consistent with direct activation of both the cyclic nucleotide-stimulated Cl^- and the NSC conductances by cAMP.

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Characterization of a non-desensitizing cAMP/cGMP gated channel on isolated human olfactory neurons

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Human olfactory neurons were isolated from excised nasal mucosa obtained from routine surgery at the middle turbinates of the nose. The isolated olfactory neurons could be identified by their characteristic morphology: the bipolar form, the presence of cilia protruding from the olfactory knob and mostly the piece of axon at the proximal end. In contrast to other species as the salamander human olfactory neurons carry only a small number of cilia. We used exised patches (in the inside out configuration) from such neurons to study channels activated from the cytosolic side of the membrane. For the application of the agonists a fast stimulation device (liqiud filament sytem) was employed. CNG channels were found to be present near the olfactory knob in low density. Both cAMP and cGMP, at micromolar concentrations, were capable of inducing channel openings. Other nucleotides as ATP or AMP were ineffective. In absence of divalent ions the single channel conductance was about 18 pS. The nearly linear current voltage relation of single channel currents with a reversal potential of 0 mV corresponds with the Nernst potential for the used Na and K concentrations. The most interesting property of this investigated channel was the absence of desensitization in the continued presence of saturating concentrations of agonists. This absence of desensitization and the selective activation by cAMP anyd cGMP give strong evidence for an intriguing similarity between the CNG channel of salamander olfactory neurons and the channel found on human olfactory neurons.

Calcium-Calmodulin Modulation of Cyclic Nucleotide-Gated Cation Channel of Olfactory Receptor Cells. K.-W. YAU, T.-Y. CHEN and M.Y. LIU. Howard Hughes Med. Inst. and Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The apparent affinity of cyclic nucleotide for the olfactory cyclic nucleotide-gated cation channel (OCNC) is reduced by Ca^{2+} in the presence of a soluble factor (Kramer and Siegelbaum, Neuron 9, 897, 1992), constituting a mechanism for olfactory adaptation. We report here that this soluble factor is likely to be calmodulin. Using excised, inside-out membrane patch recordings on both the cloned rat OCNC (rOCNC) expressed in HEK 293 cells and the native OCNC from rat olfactory receptor cells, we have found that Ca^{2+} reduces the apparent affinity of the channel for cyclic nucleotides by up to 20-fold in the presence of calmodulin. This affinity change appears to involve a direct interaction between Ca^{2+}-calmodulin (CaM) and the channel, and is blocked by the calmodulin inhibitor mastoparan. The same CaM effect was also seen for the newt and the catfish OCNCs. Using chimeric channels composed partly of rOCNC and partly of the human retinal rod cyclic nucleotide-gated channel subunit 1, which does not show any CaM effect, we have identified the cytoplasmic N-terminal segment of rOCNC to be necessary for the CaM action. Using deletion mutants of rOCNC, we have further identified a small domain on this N-terminal segment that, when deleted, results in a decrease in cyclic nucleotide affinity comparable to that produced by CaM on the wild-type channel; moreover, this mutant loses its responsiveness to CaM. Separately, gel-overlay experiments have indicated that ^{125}I-labelled calmodulin binds to a fusion protein corresponding to the N-terminal segment of rOCNC in the presence of Ca^{2+}. Inspection of the amino acid sequence of the N-terminal segment suggests the presence of a putative CaM binding site that coincides with the important domain indicated by physiological experiments. Indeed, when this putative CaM-binding site was deleted, the fusion protein no longer binds CaM. Our interpretation of the above results is that there is a domain in the N-terminal segment of OCNC important for tight cyclic nucleotide binding, but its influence becomes removed when CaM binds to it or its vicinity.

Activation Kinetics of an Olfactory Recombinant Cyclic Nucleotide-Gated Channel from Rat for Pulses with Different Second Messenger Concentrations. FRANK ZUFALL, HANNS HATT*, RODERICK V. JENSEN, GORDON M. SHEPHERD (Section of Neurobiology, Yale Medical School, New Haven, CT 06501 and *Zellphysiologie, Ruhr Universität Bochum, FRG)

In vertebrate olfactory transduction there is fundamental evidence for an odor-dependent, cAMP-gated cation channel (CNG-channel) whose activation mediates the initial large and rapid response to odor ligands. A complete understanding of the functioning of this cAMP-mediated second messenger cascade requires detailed knowledge about its kinetic behavior. Previous data using biochemical methods suggested that the odor-induced production of cAMP is rapid and transient. Initially, this result could not be reconciled with the relatively slow time course of the electrical response of olfactory receptor cells to odor application.
We therefore initiated a series of experiments in which rapid pulses of known concentrations of second messengers were applied to inside-out patches containing a cloned rat CNG-channel at high density. Agonists were applied using a piezo-switch device. We have analysed the effect of a broad range of cAMP-concentrations on both the rate of rise and the rate of decay of cAMP-gated currents. The effect of pulse duration at different agonist concentrations has also been explored. These data clearly show that gating in olfactory CNG-channels is a slow process involving a concentration-dependent binding step and a second conformational step. As a first approximation, the association rate constant for binding of cAMP to the channel was estimated from activation and deactivation current time courses to be $\leq 10^6\ M^{-1}s^{-1}$. As one of the consequences transient cAMP-pulses (10-50 ms) would lead to graded current responses for a wider range of concentrations.
In conclusion these data further support our hypothesis that channel activation is a rate limiting step in the olfactory cAMP-pathway. Therefore, in the case of rapid and transient cAMP-production, channel gating would determine the onset time course of the electrical response, giving the ion channel the role of an integrator for rapid second messenger pulses.

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Specialisation of Single Plant Odour Receptor Neurons in the Pine Weevil studied by Linked Gas Chromatography - Electrophysology

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Identification of volatiles from plants that are used as chemical cues is important in olfactory research of insects as well as other organisms. The aim of the present study was to identify plant odours used by the pine weevil, *Hylobius abietis*. This herbivore, feeding on pine and spruce, is strongly attracted by odours from their hosts. In order to identify which components of the host volatile mixtures are received by the pine weevil receptor neurons, we have linked gas chromatography (GC) to electrophysiological recordings from single receptor neurons. Volatiles from host-plant materials were trapped by headspace procedure, using Porapak Q, and eluted by hexane. These solutions were used as test mixtures. When a receptor neuron responded strongly to the mixtures, a sample was injected into the GC column. After separation the effluent was split, leading one half to the GC-detector and the other half to the insect antenna. The compounds were thus tested in sequences on the receptor neurons, allowing simultaneous recordings from the GC-detector and the receptor neuron. Successful recordings have been made from 69 neurons, most tested for several mixtures. Out of about 45 compounds of the test mixtures eliciting responses in one or more neurons, 15 have been chemically identified by GC-MS. The receptor neurons could be placed in more than 16 groups, according to which components they responded to. Selective responses to one or a few compounds were found for most neurons. Only some responded to more compounds, however, the responses were always strongest to one or two of them. Furthermore, one neuron responding selectively to one component of the host mixtures, was tested for synthetic optical isomers of that compound. In this case the neuron was strongest activated by one of the enantiomers. The results suggest that the plant odour receptor neurons in the pine weevil are rather specialist types of neurons than being broadly tuned.

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Topographical Distribution of Receptor Expressing Neurons in the Olfactory Epithelium. BREER, H., STROTMANN, J., WANNER, I., KRIEGER, J. and K. RAMING (Institute of Zoophysiology, University Stuttgart-Hohenheim), 70599 Stuttgart, FRG

Odor quality may be encoded by a spatial segregation of receptor cells with specific responsivity, determined by the distinct receptor subtypes expressed. Thus, the topographic pattern of responsivity suggests that subsets of olfactory neurons expessing a common receptor subtype may be segregated in certain regions of the epithelium. Several cDNA-clones encoding putative odorant receptors have been isolated and characterized. Digoxigenin-labelled antisense RNA transcribed from various putative odorant receptor clones was used to probe coronal sections of the rat olfactory epithelium employing in situ hybridization techniques. Initial macroscopic analysis revealed that for most of the clones reactive cells seem to be relatively wide spread; more detailed studies showed that reactive cells are restricted to certain areas of the epithelium. Some of the clones are expressed in the same region, whereas others are expressed in non-overlapping, sometimes complementary regions. The reactive areas form complementary bands extending in the anterior-posterior axis. One of the receptor subtypes was expressed only in a clustered subset of cells segregated in two very restricted areas of the olfactory epithelium. The reactive cells appear symmetrically in both nasal cavities. A detailed mapping of the expression pattern for odorant receptors may contribute to unravel the chemotopy of the olfactory system.

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PCR Amplification Of Odor Receptor cDNA From Individual Olfactory Neurons In Salamander.
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The olfactory receptor multigene family is composed of hundreds of genes encoding a large diversity of odorant receptors, suggesting that odor recognition may rely heavily on the discriminatory capabilities of these receptors. Electrophysiological recordings have shown that single olfactory neurons are often responsive to more than one odorant. This could occur in either of two ways. For one, each cell may express multiple members of the odor receptor family, each receptor being specific for a particular odor. Alternatively a cell may express a single type of odor receptor that is broadly sensitive to several odorants. To determine which of these strategies olfactory neurons have adopted, and to obtain the relationship between olfactory receptors and odorants, we have developed a procedure for PCR amplifying members of the olfactory receptor family from the cytoplasmic contents of single olfactory neurons after obtaining patch clamp recordings of their responses to odors.

Freshly dissociated salamander olfactory neurons were chosen for recording the response to odors by the whole-cell patch clamp technique. Under visual control negative pressure was applied and the cytoplasm containing the receptor mRNAs was collected into the recording pipette and transferred into a microcentrifuge tube. The nucleus of the cell was not harvested to avoid genomic DNA contamination. The complementary DNA of mRNA was firstly synthesized in the tube, and the target cDNA was then amplified by PCR. The degenerate oligonucleotide primers for the PCR were designed based on the known sequences of the 3rd, 6th and 7th transmembrane regions of putative olfactory receptors in rat. Using these primers we were able to isolate about 20 new PCR products representing putative olfactory receptors from salamander olfactory epithelium cDNA. The PCR products from single cells were subcloned and several clones from each cell were randomly chosen for sequencing. Among the first 20 olfactory neurons we analyzed 5 showed PCR products of the proper size after amplification using degenerate primers corresponding to the 3rd and 7th transmembrane regions. Three of the products were determined to be members of the odor receptor family based on their sequence homology.

Although preliminary, these results indicate that it is possible to reliably amplify and identify the odor receptor mRNA contained in a single olfactory neuron after recording its physiological response to odors.
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Members Of A Family Of Drosophila Putative Odorant-Binding-Proteins Are Expressed In Different Subsets Of Olfactory Hairs. C. W. PIKIELNY (Brandeis U.), G. HASAN (Indian Instit. of Sciences), F. ROUYER (Brandeis U.) and M. ROSBASH (Brandeis U.).

We have developed a PCR-based method allowing the construction of subtractive cDNA libraries starting with very small amounts of RNA (nanograms). Using this method, we have generated a cDNA library from the antennae (the main olfactory organ) of *Drosophila melanogaster* from which head cDNAs have been subtracted. Among the clones in this library we have found apparent *Drosophila* homologues of two enzymes proposed to be involved in odorant inactivation in vertebrates, cytochrome P450 and UDP-glucuronosyl transferase. Their specific (UDPGT) or preferential (P450) expression in antennae implies a role in olfaction conserved throughout evolution. We have also found a number of clones that correspond to small, abundant, antennal-specific mRNAs. Strikingly, *in situ* hybridization with seven of these clones shows that each is expressed in a different subset of olfactory hairs. Five encode proteins similar in sequence to moth pheromone-binding-proteins; PBPs are abundant proteins secreted in the aqueous medium bathing olfactory neurons in the lumen of pheromone-sensing hairs. Another is similar to vertebrate proteins that bind small, hydrophobic ligands. In all cases, a putative signal peptide can be found at the amino-terminal end. Our results suggest the existence of subsets of olfactory hairs, each containing different secreted proteins in their internal aqueous medium. We speculate that these proteins may be odorant binding proteins and that their segregation in different olfactory hairs may reflect the odor specificity of these hairs.

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Perception of Mixtures of Airborne Chemicals by the Olfactory, Nasal Trigeminal, and Ocular Trigeminal Sensory Systems.
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Over the past few years we have measured thresholds of odor (in normosmics), nasal pungency (in anosmics), and eye irritation for a variety of relatively non-reactive volatile organic compounds (typically, members of homologous series: alcohols, acetates, ketones, alkylbenzenes). Olfaction proved always the most sensitive modality. In every series, all three sensory thresholds declined with carbon chain length, but only common chemical sensations (nasal pungency and eye irritation) displayed a uniform relationship with simple physicochemical properties (e.g., saturated vapor concentration) across the various chemical families. This led us to conclude that the reception process in the common chemical sense (CCS) relies on a broadly tuned physicochemical interaction between stimulating molecules and the receptive biophase. In the present study, we measured odor, nasal pungency, and eye irritation thresholds for mixtures of some of the previously studied individual substances. On the basis of previous thresholds for single compounds, we prepared five mixtures (two three-component, two six-component, and one nine-component mixture) where the constituents were at equivalent sensory potency (i.e., all at threshold, or all at the same multiple or sub-multiple of it). Again, odor thresholds fell below the other two. All thresholds decreased as the mixtures gained complexity, implying additivity of the sensory effects of individual components. Eye irritation even showed indications of synergism in two of the more complex mixtures. For mixtures of equal complexity (i.e., equal numbers of components), those made of relatively lipophilic (as opposed to water-soluble) substances displayed more additivity of eye irritation and nasal pungency, but not of odor. Overall, our results at threshold levels suggest that the CCS, although operating at higher concentrations than olfaction, integrates the signal from individual components of chemical mixtures more completely than olfaction.

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Cognitive-, Facial-, and Heart-Rate Responses Equally Indicate Taste- and Odor- Hedonics.

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Our previous studies revealed that facial displays, evoked by tastes and odors, are reflecting both hedonics and intensity of sensations in neonates. Later, we have demonstrated that rating of such facial behaviors can be utilized in sensory-testing of adults too, corresponding in reliability with psychophysical scaling. Other observations showed, that stimulus-induced transient changes of heart- and respiratory-rate (HR.; RR.) can also indicate hedonics of chemosensations. The present study aimed to assess correlation between subjects' psycho-physical self-estimates and observers' ratings on testees' facial displays. Further, correlation between cognitive scaling and stimulus-induced HR.- and RR.- changes was also studied. 80 healthy young volunteers of both sexes were presented with self-administered 5 intraoral and 4 nasal stimuli. Their face was videorecorded: at rest, during stimulaion and for a 1 min. post-stimulus follow-up, simultaneously with a digital HR. and RR. monitor. A 100 mm visual analog scale served both testees and observers for hedonic and intensity estimates. Results show: a) Trained and naive observers agree in rating hedonics, expressed in stimulus-induced facial displays (r>0.8). b) Hedonic and intensity self-estimates and the obervers' ratings of facial displays, were also found to be in close correlation (r>0.8). c) Stimulus-induced transient HR-increment was lowest in response to tastes and odors rated as "indifferent", highest for those rated as "aversive";"pleasant" ones induced interim changes. d) RR-changes were found to reflect less clearly hedonics than do HR changes. These findings render GUSTO-and NASOFACIAL REFLEXES as well as HR- changes as alternatives to cognitive psychophysical tests.

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Influence of Oral Volume Capacity on Perception of Astringent Intensity

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The detection of astringency involves a mechanical process that requires manipulation of the tongue against oral surfaces. Drying and roughing are perceived due to the precipitation of salivary proteins, de-lubricating oral surfaces; the aggregate precipitation is dependent on the concentration of the stimulus and the amount of protein in the mouth. Since the protein sources theoretically include both the saliva and the oral epithelium, the size of the oral cavity may be a factor in the perception of astringent intensity. The total available area may affect the magnitude of astringent sensations, producing different impressions among the consumers of astringent foodstuffs. Measurements of the maximum amount of water subjects could hold in their mouths, the amount that constituted a "sip" and their intensity ratings of astringent solutions were submitted to multiple regression analysis and Pearson's product-moment correlation. Correlation coefficients showed an inverse relationship between the amount of water held in the mouth and the level of intensity perceived, for some subjects. Dividing the measurements into groups according to gender revealed that males had significantly higher volumes for the maximum amount of water that could be held in the mouth and for a "sip". Correlations between intensity perception and the potential contact areas of the oral cavity suggests that oral surfaces play a role in astringency. Diminishing intensity ratings according to mouth size are consistent with the view that astringency is a tactile sensation. If ranges in mouth size modify the perception of intensity because astringency relies on the available surface area for protein sources, as well as the proteins in the saliva, then this factor must be considered when interpreting results of astringency studies.

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Effects of oral irritation on components of flavour mixtures

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Recent studies show that the oral irritant principle in chilli, capsaicin, will suppress taste intensity but that this depends on both the taste and the relative intensities of tastant and capsaicin. To date there has been no research reported on how irritants affects flavour intensity and the taste and odour components of the flavour. Subjects were asked to rate the intensity of the overall flavour and odour and taste components of mixtures of (a) orange flavour, citric acid and sucrose, and (b) vanilla flavour, citric acid and sucrose, to which capsaicin at 1, 4 and 16 ppm had been added. Subjects received all combinations of capsaicin and flavour mixture with a minimum of 48 hrs between each solution. Ratings were made while the mixture was in the mouth and, following expectoration, at 20 sec intervals for 3 mins. Two groups of subjects, frequent and infrequent chilli users, completed the experiment. The effects of capsaicin on flavour mixtures as a function of subjects' frequency of chilli consumption and the degree of congruity of the flavour mixture components will be discussed.

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Matching and Scaling of Taste-Smell Mixtures: Individual Differences in Sweetness Enhancement by Strawberry Odor.

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Previous research has indicated that taste-smell interactions depend on the tastant, the odorant, and the task at hand. When subjects are forced to judge one dimension of a complex taste-smell mixture, subjects overestimate the intensity of the target taste quality in certain mixtures. This enhancement disappears when subjects are allowed to make judgments along several, more appropriate response scales. Since it can be argued that the observed enhancement may be caused by scaling biases due to the use of category rating scales, a non-metric method was used. Thirty subjects judged 0.18, 0.30 and 0.435 M sucrose, and 0.30 M sucrose mixed with 1% strawberry odor or 0.1% coffee odor. The five stimuli were presented in two random blocks using a scaling task, and in two random blocks during a matching task. The order of the tasks was counterbalanced. During scaling, subjects were instructed to rate the sweetness of the stimuli along a 21-point category scale, ignoring all other sensations. During the matching task, they were asked to indicate that solution from a range of nine sucrose solutions (0.13 to 0.55 M) that best matched the sweetness of the test stimulus. The results indicated that strawberry odor significantly enhanced the perceived sweetness of sucrose, using both the matching and the scaling task. Subjects judged the mixture to be more than 1 concentration step (matching) or 2 units (scaling) sweeter than unmixed sucrose. Coffee odor also significantly enhanced sweetness, but to a lesser degree. Individuals clearly differed in their tendency to enhance, but were consistent across methods. This was confirmed by high correlations between the individual enhancements scores during scaling and matching, for strawberry (r=0.50) and coffee (r=0.43). We hypothesize that cognitive processes underlie taste enhancement by odors. Some individuals ignore background dimensions of complex stimuli, while others combine related dimensions.

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Taste and oral sensations elicited by benzoic acid derivatives

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Sensory properties of equimolar concentrations of benzoic acid derivatives were examined using Time-Intensity procedures. Significant differences in maximum intensities ($P<0.005$) were found for astringency, bitterness, prickling, sourness and sweetness. Although these compounds differed only in the number and position of the hydroxy groups, they exhibited quite different profiles. Gentisic acid had the highest sourness and bitterness maximum intensity, salicylic and gentisic acids were highest in astringency, and m-hydroxybenzoic acid was the sweetest sample. Benzoic acid had the highest intensity of prickling feeling which lasted 20 sec longer than that of salicylic acid and 40 sec longer than the other samples which elicited little prickling feeling. In principal component analysis of maximum intensity data, the first factor contrasted bitterness and sourness vs sweetness; the second discriminated benzoic and salicylic acids, which were highest in prickling from samples which were low in this attribute. Protocatechuic and gallic acids were closely clustered reflecting their similar sensory properties. Although masking and interaction effects complicate the interpretation, the effect of chemical structure or properties on perceived sensory attributes will be discussed.

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NADPH Diaphorase Activity in Olfactory Receptor Neuron Axons in Hamsters Conforms to a Rhinotopically-Distinct Dorsal Projection Zone in the MOB.

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NADPH diaphorase histochemical activity is distributed widely in the CNS, where it is most commonly localized to short-projecting neurons whose axons do not collect, for the most part, in the major white matter tracts such as the corpus callosum or cerebral peduncles (Vincent and Kimura, 1992, Neuroscience, 46:755). By contrast, the olfactory and vomeronasal nerves show intense, locally diffuse NADPH diaphorase activity in the main olfactory bulb (MOB) and accessory olfactory bulb (AOB), respectively. Nevertheless, the olfactory nerve activity patterns are also topographically restricted to the dorsal and medial MOB (Scott et al., 1987, J. Comp. Neurol., 260:378; Croul-Ottman and Brunjes, 1988, Brain Res., 460:323; Davis, 1991, J. Comp. Neurol., 314:493). When examined across a full series of coronal sections and compared with recently developed maps of the rhinotopic organization of primary olfactory projections to the MOB (Schoenfeld et al., Brain Res. Bull., in press), the topography of NADPH diaphorase activity in the MOB conforms almost precisely to an obliquely oriented, rhinotopically-distinct dorsal zone known to receive projections from mucosal segments that line a relatively smooth central channel within the nasal cavity (Clancy et al., Brain Res. Bull., in press). The NADPH diaphorase negative regions of the ventral and lateral MOB conform, on the other hand, to a rhinotopically-distinct ventral zone known to receive projections originating from more convoluted channels situated ventral, lateral and dorsal to the dorsally-projecting central channel. Common characteristics between the dorsally-projecting central channel and the vomeronasal organ may provide clues to explaining the comparable NADPH diaphorase activity found in their bulbar projections.

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NADPH-diaphorase Histochemistry in the Nasal Chemosensory Systems of Immature and Adult Opossum, *Monodelphis domestica*.

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Nitric oxide (NO) is thought to play important roles as a second messenger and a neurotransmitter in several systems of the brain. NADPH-diaphorase histochemistry, a marker of the synthesizing enzyme of NO, nitric oxide synthase (NOS), provides a simple, albeit indirect, method for studying this novel messenger. Although it is now well accepted that the NADPH-dependent reaction product, presumably NOS, is localized in the nasal chemosensory systems, few studies have described its localization pattern during development in these systems. We examined the developmentof NADPH histochemistry in the olfactory and vomeronasal systems of opossums, *M. domestica*, since these marsupials are born in an embryonic state of development. 15-, 45-day-old, 2-, 3-, 4-, 5-month-old, and adult opossums were used. Since few differences were observed from 2-month-old to adult, these groups are discussed together. At 15 days of age, dark blue NADPH reaction product was observed at the lumenal surface of the olfactory and vomeronasal epithelium, in olfactory (Bowman) and vomeronasal glands, and at the surface of the olfactory ventricle. Some light staining was also observed in blood vessels throughout the brain. At 45 days of age, dark staining was still observed at the lumenal surface of the epithelia and throughout the glands (the cell bodies and axons of bipolar neurons were clearly unstained). In the brain, olfactory ventricular staining was still evident. In the main olfactory bulb (MOB), the olfactory nerve layer (ONL) was clearly unstained, whereas glomeruli were differentially stained: some lightly-stained glomeruli were scattered among more darkly stained ones. Most interestingly, the accessory olfactory bulb (AOB) was also differentially stained: the rostral part of the glomerular layer (GL) was darkly stained, whereas there was very light to no staining in the caudal half. However, periglomerular cells were darkly stained throughout the rostro-caudal extent of the AOB GL. In animals 2 months of age and older, this pattern of staining remained, except for an apparent lack of ventricular staining in the MOB. The differential pattern of AOB staining in opossums compliments our previously described results with antibodies to OMP, the VVA lectin, and with antibodies to G proteins (Shinohara et al., unpublished observations) and suggests that the AOB is a structurally and, perhaps, functionally heterogeneous structure.

Localization of Nitric Oxide Synthase in the Olfactory Epithelium of the Rat and Channel Catfish.

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Stimulation of olfactory neurons with odorants results in a rapid transient elevation in the levels of either cAMP or $InsP_3$ followed by a slower steady increase in cGMP concentration. It has been proposed that the increase in cGMP formation is mediated by stimulation of guanylyl cyclase by nitric oxide (NO), a simple gas that serves as an unorthodox neurotransmitter in the central nervous system (Breer and Shepherd, TINS 16:5-9). NO is produced as a byproduct of the reduction of L-arginine to citrulline catalyzed by the enzyme nitric oxide synthase (NOS), which requires NADPH as a co-factor. NOS can be localized histochemically by using an NADPH diaphorase histochemical technique in which nitroblue tetrazolium is reduced to formazan forming a blue precipitate in NOS-positive cells. Using this technique, we have localized NOS to olfactory neurons and nerve tracts in rat and catfish olfactory epithelia. In the rat staining was also found in bottle-shaped cells with morphology reminiscent of microvillar olfactory cells. In contrast, the respiratory epithelium and the sustentacular cells in the olfactory epithelium displayed little staining. The NADPH diaphorase reaction was stimulated by addition of excess L-arginine and was inhibited by L-N^G-nitro arginine (NO_2Arg), a blocker of NO production indicating that the staining was specific for NOS. When the NADPH diaphorase reaction is made non-specific by prolonged incubation, the reaction product was found in all cells and was not inhibited by NO_2Arg. Unilateral bulbectomy, which causes degeneration of mature olfactory neurons on the bulbectomized side, markedly reduced NADPH diaphorase staining. Biochemical assays, based on the enzymatic conversion of L-arginine to L-citrulline, verify the presence of NOS in the rat and catfish olfactory epithelium. These results are consistent with a role for NO in olfactory transduction.

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Heme Oxygenase-2 in the Olfactory System: Localization, Correlation with Cyclic GMP Levels and Regulation of Activity by TGF-beta.

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Recently, evidence has suggested that carbon monoxide (CO) may serve as an endogenous intercellular messenger. High levels of heme oxygenase-2 (HO2), the enzyme which degrades heme to biliverdin and generates CO, are found in the olfactory epithelium. Previously, we have shown that olfactory receptor neurons (ORN's) in culture have high HO2 activity. We now demonstrate by immunohistochemistry that HO2 is localized to olfactory cilia. CO may activate guanylyl cyclase (GC) similar to nitric oxide. Metabolic labeling experiments have been devised which permit the direct measurement of labelled CO in ORN's *in vitro*. These results demonstrate that cGMP levels parallel CO production, and that inhibitors of HO2 reduce cGMP levels and CO levels. This suggests that in ORN's, CO may regulate cGMP levels. TGF-beta, but not other growth factors, causes a drop in CO production , providing a mechanism whereby TGF-beta may act.

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Localization of NADPH-Diaphorase Activity in *Drosophila* Olfactory Tissues

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Recent evidence suggests that nitric oxide acts as an intercellular messenger in vertebrates. With few exceptions, nitric oxide synthase (NOS) colocalizes with NADPH-diaphorase (NDP) . NOS catalyzes the conversion of L-arginine to L-citrulline and nitric oxide (NO), a free radical gas. Frozen sections of wildtype *Drosophila* heads were stained for NDP activity. Strong staining was detected in antennae and maxillary palps, which are the adult olfactory organs. In the brain, staining is detected in some of the glomeruli of the antennal lobes. Staining is also observed in mushroom bodies, which have been implicated in olfactory learning and memory. Interestingly, strong staining is detected in the labellum, a taste organ. The labellum is covered with taste bristles, each of which is innervated by a number of chemosensory neurons and a single mechanosensory neuron. Cell bodies and axons underneath most if not all taste bristles are stained. This NDP staining is strongly inhibited in the presence of N^G-methyl-L-arginine, suggesting that the staining is specific for nitric oxide synthase.The lozenge (*lz*) mutant of *Drosophila* completely lacks one type of olfactory hairs, the sensilla basiconica, in the antennae. In this mutant sensilla basiconica are also defective in the maxillary palp. NDP staining is greatly reduced in the antennae, maxillary palps and brain of the lozenge mutant, consistent with the possible involvement of the NO pathway in *Drosophila* olfaction.

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Expression of Nitric Oxide Synthase in Olfactory Epithelium and Bulb, and in the Vomeronasal Organ and Accessory Olfactory Bulb of Rats: Variation With Age. LINDA M. WYSOCKI, CHRISTIAN DELLACORTE and CHARLES J. WYSOCKI (Monell Chemical Senses Center, Philadelphia, PA)*

Nitric Oxide, a gaseous neurotransmitter within the CNS, is produced as a by-product of the reduction of L-arginine to citrulline catalyzed by the enzyme nitric oxide synthase (NOS). NOS can be localized with NADPH diaphorase histochemistry. We so treated tissue obtained from embryonic, neonatal and adult rats; NOS was detected in the embryonic olfactory epithelium. Morphologically, NOS-positive cells were diverse. Many cells appeared bipolar, with a clear olfactory knob protruding from the apex; their somata could be found at various depths in the epithelium. Other cells were more spherical or basket shapped; they appeared to be restricted to the surface layer of the epithelium. Density of NOS-positive cells, distributed throughout the rostral/caudal extent of the epithelium, increased with age. NOS-positive cells were not detected within the embryonic vomeronasal organ (VNO) nor were NOS-positive fibers/glomeruli noted within either the main (MOB) or accessory olfactory bulbs (AOB). NOS-staining of tissue from neonatal rats differed from that of the embryos. Olfactory neurons continued to be NOS-positive, but by 2 days of age, NOS-positive cells were noted in both the neural and non-neural epithelia of the VNO. Olfactory axons were now NOS-positive and many golmeruli within the MOB (but not AOB) were clearly defined by NOS-positive fibers. In tissue from adults, NOS-positive bipolar cells were readily visible in the epithelia. There were numerous NOS-positive glomeruli within the MOB and the glomerular and vomeronasal nerve layers of the AOB were heavily stained. Functional correlates between age and variation in NOS staining and an understanding of the differences between the olfactory and vomeronasal systems require additional investigations.

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Nitric Oxide Synthase Staining in the Lobster Olfactory System. A. BARNHART and E. ORONA. (Whitney Laboratory, University of Florida, St. Augustine, FL. 32086)

Nitric oxide has recently been implicated as a potentially important signal mediator in the primary receptor cells of the mammalian olfactory system, although its ubiquity in invertebrates is virtually unexplored. Here we report on the localization of putative nitrergic cells by staining for nitric oxide synthase (NOS) in the lobster olfactory system, using the histochemical reaction for β-nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase. NADPH staining was performed on wholemounts of the lobster antennule (olfactory organ), bearing the somata and dendrites of the primary receptor cells, and on brain sections, containing the somata and neuropil of the olfactory lobes. In the antennule, the somata of the receptor cells exhibited very light or moderate staining for NADPH, although the dendrites of the cells demonstrated specific staining. In the brain, NADPH staining was intense throughout many neuropils, including the olfactory lobes (OLs) where the primary afferents terminate. The olfactory glomeruli were intensely staining, and the staining in the cup/subcap regions of the glomeruli was especially dense. The second-order olfactory neurons which innervate the OLs are found primarily in the lateral and medial clusters (LC, MC) adjacent to the OL. No somata in the LC demonstrated NADPH staining. However, several small and large somata in the MC contribute to the staining pattern found in both the OL and AL (accessory lobe). These results suggest that nitric oxide may function as an important signal molecule in the primary and secondary levels of the lobster olfactory pathway.

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Immediate-Early Gene Expression in the Regenerating Olfactory System of the Spiny Lobster. E. ORONA and A. BARNHART. (Whitney Laboratory, University of Florida, St. Augustine, FL. 32086)

Members of the "immediate-early" gene (IEG) family, such as c-*fos* and c-*jun*, function as inducible transcription factors in stimulus-response coupling, and their expression has been correlated with cellular differentiation and development. Understanding the functional organization of the olfactory system is complicated because of the unusual capacity of the primary receptor cells to regenerate. Since there is a presumed specificity in the axonal projections to the CNS encoding odor quality, how the functional constancy in this projection is maintained is unknown. In addition, little is known about the nuclear molecular genetic mechanisms of damaged neurons that initiate and maintain axonal ingrowth and sprouting. Here we report on the induction of Jun-like immunoreactivity, produced by deafferentation of the primary olfactory axons to the brain of the spiny lobster. Expression of IEG family members was investigated using various antisera against the protein products, applied to brain sections and in Western blots of central and peripheral tissue extracts. Removal of the olfactory organ (antennule) resulted in an induction of Jun-like immunoreactivity (a 39kD protein) in the lobster brain. This effect occurs several hours following deafferentation, and persists for 1-2 wks. The enhanced gene expression was specific to a c-jun antiserum against the N-terminal, but not to c-jun antiserum against the DNA-binding domain. JunD-immunoreactivity was also enhanced in these deafferented brains, but no effects were observed with antisera against JunB or c-fos. This induction was also observed in Jun-like staining in somata of second-order olfactory neurons, innervating the olfactory lobes. In the periphery, enhanced IEG expression was *not* observed in Western blots, using antennular tissue innervating aesthetasc sensilla. In crustaceans, the "oldest" antennular segments are located distally, and receptor cells are generated anew toward the proximal end of the antennule, behind the segments actually bearing aesthetasc sensilla. Remarkably, when tissue from this region is immunostained, intense Jun-like immunoreactivity *is* observed, suggesting the detection of progenitor cells of olfactory receptor neurons. This induction of IEG expression during regeneration of peripheral and central olfactory structures, and the functional dissociation between *fos* and *jun* expression, parallel similar phenomenon in mammals.

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Life-Stage Changes in the Neuroanatomy of the Salmon Olfactory Bulb: Implications for a Peripheral Component in Olfactory Imprinting.
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The Chinook salmon (*Oncorhynchus tshawytscha*) is known to imprint to the odor of its home-stream at about 1.5 yrs of age during a period of morphological, physiological, and behavioral change known as parr-smolt transformation. This retained odor memory is later used by the adult during the spawning migration as a homing cue to the natal stream. Using an immunocyto-chemical technique that selectively labels peripheral olfactory afferents (Riddle and Oakley [1992], J.Comp.Neurol., 324:576-89), we have begun analyses of salmon olfactory bulb components at different life stages to characterize changes in the bulb across the parr-smolt transformation. In preliminary data, we have compared the relative areas of the glomerular layer (GL) and inner-cell layer (ICL) of the bulb at analogous mid-dorsoventral horizontal sections for parr (n=4), postsmolt (n=1), and adults (n=4). Data normalized to olfactory bulb area shows a 13% increase in GL area over the course of parr-smolt transformation. Between postsmolt and adult stages, however, the GL shows a 7.4 % decrease in relative area . The ICL shows a consistent decrease of 8% and 1% over these two periods. These preliminary results are consistent with and expand upon previous work in the Atlantic salmon (Bowers, '88, unpubl. dissertation, Yale Univ.). Results suggest that there is an increase during parr-smolt transformation in peripheral olfactory receptor cells (ORC), whose projections make up the GL. A selective proliferation of ORCs sensitive to home-stream odors may be involved in olfactory imprinting.

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Changes in Expression of Fos Protein In the Developing Rat Olfactory Bulb Following Unilateral Naris Occlusion. ANNA Y. KLINTSOVA, BENJAMIN D. PHILPOT, PETER C. BRUNJES (University of Virginia).

The *c-fos* immediate early gene exhibits regionally-specific patterns of expression during early development (e.g., Alcantara and Greenough, *J. Comp. Neurol.*, **334**, 1993). Adult levels can be altered by a variety of manipulations (e.g., sensory stimulation or deprivation, learning, and drug application). In the present study, temporal and spatial patterns of Fos protein immunoreactivity were examined in the developing olfactory bulb via avidin-biotin peroxidase immunocytochemistry (Fos antibody kindly provided by Dr. Frank Sharp, University of California, San Francisco). Tissue from both control rats and animals with a single external naris surgically sealed on the day after birth (P1) was examined. Bulbs were studied at 2, 12 and 24 hr following occlusion, as well as at P15, P20 and P30. In tissue from control animals in the 2-24 hours group, staining was localized to the cytoplasm of mitral and granule cells as well as their dendrites. Immunoreactivity was localized to nuclear regions in P15, P20 and P30 pups. Fos-immunopositive nuclei were most dense in rostro-lateral portions of the bulb, primarily in the granule cell layer. Intermediate levels of staining were observed in medial bulb areas. Naris occluded pups exhibited normal patterns of immunolabeling in the first 24 hr. However, staining was absent or sparse in the bulb ipsilateral to the occlusion at older ages, suggesting radical alterations in fos expression. Levels of Fos staining in the contralateral bulb of occluded animals did not differ from controls. Studies are underway to determine when alterations in staining between normal and experimental bulbs first emerge.

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DevelopmentofOligodendrocyte/Myelin-Immunoreactivity in the Olfactory Bulb. BENJAMIN D. PHILPOT, ANNA Y. KLINTSOVA, and PETER C. BRUNJES (University of Virginia).

During early life, oligodendrocytes, migrate, differentiate, secrete growth factors, and eventually extend processes that form myelin sheaths. The present study used ABC immunocytochemistry to visualize oligodendrocyte/myelin-immunoreactivity in the developing olfactory bulb. Postnatal Day 10 (P10), P15, P20, P30, and adult rats were examined, as well as P20, P30, P40, and adult *Monodelphis domestica* (the grey, short-tailed opossum). In the rat, immunoreactivity first appears in the accessory olfactory bulb at approximately P10, with staining rapidly increasing throughout the entire bulb over the next five days. By P20 immunostaining assumes an adult distribution, characterized by dense staining in submitral areas, sparser labelling in the external plexiform layer, and staining along the periphery of glomeruli. Staining density increases from P20 to adulthood. Surgical closure of one external naris on P1 had little effect on developing patterns of staining. In the opossum, no staining was observed in the bulb until P30, although corticospinal myelination is evident by P20. Adults of both species exhibit quite similar patterns of immunostaining. The present study suggests that myelination first begins in the accessory olfactory bulb and then rapidly proceeds to the main bulb. It also indicates that *Monodelphis* is a favorable species for studying myelination as oligodendrocyte maturation occurs over an extended period, allowing increased resolution into early development.

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Cross-strain Transplantation of Olfactory Bulbs in Rat. MARY E. LEE (University of Washington), JON N. KOTT (University of Washington), LESNICK E. WESTRUM University of Washington), RAYMOND D. LUND (University of Cambridge).

We have previously shown that olfactory bulb (OB) transplants (TX) into rat hosts of the same strain survive, are reinnervated and become connected with host brain, whereas mouse OB TXs placed in rats are rejected. In an attempt to evaluate the usefulness of cross-strain TXs we are transplanting OBs from Long-Evans (LE) rat fetuses into Sprague-Dawley (SD) rat hosts of similar ages to our previous studies; and then, by using a skin-patch technique, we are studying the characteristics of the TX OB after induced rejection. Time-mated LE dams received injections of tritiated thymidine on embryonic days (E) 12-14. OBs from LE fetuses (E14-15) are immediately transplanted into newborn SD rats in the site from which the host OB was removed. In order to study the developmental aspects of the cross-strain TX at ages 2 weeks and later, sagittal frozen sections of forebrain were stained for cells and fibers or immunoreacted for olfactory marker protein (OMP*), the latter a label for mature primary olfactory nerve fibers. In a second group of 6 weeks and older, a $1cm^2$ patch of skin taken from the flank of a LE rat was placed on the flank of each SD rat that received a cross-strain TX as a neonate. At 1 week post-skin-patch and later, sagittal frozen sections of forebrain were stained for cells, fibers, and degenerating terminals or immunoreacted for OMP. Autoradiography was carried out on alternate series of sections in both groups of rats to identify the donor OB TX. The LE OB TXs survive in a high proportion of SD hosts, are attached to the olfactory peduncle and are robust, often near the size of the normal OB. The cytoarchitecture is somewhat disorganized as in SD-SD TXs, but autoradiography demonstrates large numbers of heavily labeled donor cells of various sizes. OMP material shows primary olfactory nerve fibers innervating the LE OB TX whereas the cell-fiber stains show continuity between the TX and the ventral forebrain. In skin-patch animals, fiber stains show mild to heavy degeneration in the OB TX and in the ventral forebrain, and the Nissl stain shows lymphocyte infiltration of the OB TX in varying degrees. These results indicate that cross-strain transplantation in this system is successful, and that an immunological attack on the donor tissue can be induced using a skin-patch method.

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Effects of unilateral olfactory deprivation on olfactory bulb responses to odors in the rat.
DONALD A. WILSON (University of Oklahoma)

Unilateral olfactory deprivation in rats produces marked structural changes in olfactory bulb neuroanatomy. As with many other sensory systems, the magnitude of these changes are dependent on the age at onset and duration of the deprivation. The physiological consequences of olfactory deprivation, however, are less well understood. Deprivation starting at PN1 and lasting 20 days produces a hyper-responsiveness of mitral/tufted cells to odors after reopening of the sealed naris, and an enhancement of interneuron-mediated inhibition. Late onset deprivation has less of an effect on inhibition, however, the effects on odor responses have not been thoroughly studied. Therefore, the present study examined the effects of late onset olfactory deprivation on olfactory bulb responses to odors. Wistar rats had a single naris sealed on PN30. After 1-12 months of deprivation, mitral/tufted cell single-unit responses to odors (citral, peppermint) were examined in the deprived bulb. The rats were anesthetized with urethane, the sealed naris was reopened and the contralateral naris was sealed. The results suggest that the deprived olfactory system maintains its responsiveness to odors even after long-term deprivation, and in fact, demonstrates an enhancement in responsiveness. Preliminary 2-deoxyglucose studies suggest that this enhanced responsiveness may be due (in part) to the deprivation-induced decrease in glomerular layer dopamine activity.

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Depression Affects Olfactory Recognition
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There is a suggestion of an association between olfaction and emotion. The olfactory system has connections to the limbic system, structures which facilitate memory and emotions, including the amygdala and hippocampus (Price, 1987). In fact, the odor experience can evoke nostalgic and emotional memories (Engen, 1991). The present study examined the relationship between olfaction and depression. Depressed patients often describe changes in appetite, weight, and olfactory and gustatory sensitivity (Cleghorn and Curtis, 1959; Harris, Young and Hughes, 1984; Lewis, 1934). However, olfactory memory has not been found to differ between high and low trait depression groups (Settle and Amsterdam, 1991). Work thus far a) has been limited to clinical populations and b) has failed to examine fluctuations in patients' current emotional states. The present investigation is an attempt to examine odor recognition ability in a population of college undergraduates, correlated with their current mood state. Sixty-four subjects were administered the Zung depression scale (trait depression) and a modified version of the Zung designed to examine the subject's depression at the time of testing (state depression). All subjects were given a thirty item odor list and a forty-five item yes/no recognition task. Effects of depression on the odor task were seen at the state level. State depression was found to interact with the subject's trait depression, producing decremental effects for high trait depressed individuals.

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Expression of *Pax-6* in the Developing Olfactory System of *Xenopus laevis*. JENNIFER J. SWIERGIEL, KAREN K. OISHI, and GAIL D. BURD. (Dept. of Molecular and Cellular Biology, University of Arizona, Tucson, AZ.)

Pax-6 belongs to a family of proposed developmental regulatory genes that is present in organisms as diverse as zebrafish and humans. *Pax-6* has homology to known transcription factors; it contains both paired box and homeobox DNA binding motifs. This highly conserved gene participates in the development of the olfactory and visual systems in mice and humans; olfactory and visual system defects in *sey* mice and humans with aniridia can be traced to various defects in the corresponding *Pax-6* gene and its expression. We are interested in studying the role of *Pax-6* in the developing olfactory system of the African clawed frog, *Xenopus laevis*. In order to do this, we isolated and sequenced the *Xenopus Pax-6* cDNA. Examination of the sequence revealed amino acid similarity ranging from 96%-100% in the DNA binding regions when compared to the *Pax-6* sequence of zebrafish, mice and humans. Overall amino acid similarity ranges from 90-92%. *In situ* hybridization revealed expression of *Pax-6* in the developing olfactory system; *Pax-6* is expressed in the olfactory placode, the developing olfactory epithelium and olfactory bulb, as well as in other brain areas.

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Serotonergic Receptor Involvement In Conditioned Olfactory Learning in Neonatal Rats.

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We have recently shown that serotonergic input to the olfactory bulb is necessary for the acquisition and/or retrieval of a conditioned olfactory memory in newborn rats (McLean et al., 1993). We are currently trying to determine more precisely the role of 5-HT in this learning process. Injections of different doses (1nM-10μM) of ritanserin, a 5-HT_{2a} antagonist, directly into the olfactory bulb just before odor/stroke training resulted in lost preference for a learned (peppermint) odor at lower doses. The decrease in preference did not appear to be as complete as when serotonergic input to the bulb was totally eliminated suggesting that other 5-HT receptor subtypes, in addition to 5-HT_{2a}, may be involved in the learning. We also injected ritanserin (2.5mg/kg) or saline subcutaneously into PND 7 pups before or after odor/stroke training. When pups were tested for olfactory preference on PND 8 the results showed that giving ritanserin before training blocked learning (ie. preference for the peppermint), just as we had found with direct injections into the bulb. Ritanserin injections 10 or 30 min after training did not differ from saline-injected controls. In conclusion, it appears that the 5-HT_{2a} receptor in the olfactory bulb is involved in the acquisition of associative learning but may not be involved in the retrieval of memory stores.

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Locus coeruleus modulation of olfactory-based behaviors in newborn rats.

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Olfactory bulb norepinephrine (NE) is necessary for associative olfactory learning in newborn rats. Infusion of propranolol into the olfactory bulb during associative training blocks acquisition of a learned odor approach response. The primary source of olfactory bulb NE is believed to be the locus coeruleus (lc). The present study examined the effects of lc lesions and lc stimulation on olfactory learning in rat pups. EXPERIMENT 1: On PN4, pups were anesthetized and received bilateral infusions of 6-OHDA into the lc or vehicle infusions. On PN6, pups were trained in a classical conditioning paradigm (UCS= intraoral milk; CS= citral odor) in either PAIRED, ODOR-only, MILK-only or BACKWARD (milk then odor) conditions. On PN7, acquisition of a learned odor preference to the CS was tested in a two-odor choice test. Following the test, olfactory bulbs were removed for HPLC analysis of monoamine content. Pups receiving lc lesions did not appear to differ in behavioral response patterns during training compared to their littermate, vehicle controls. However, lc lesions impaired acquisition and/or expression of early olfactory memories. EXPERIMENT 2: On PN5 pups were implanted with chronic bilateral cannulas aimed at the lc. On PN6, pups were exposed to citral during activation of the lc with infusions of the alpha-2 antagonist idazoxan (0, 1 or 2 uM). Preliminary results suggest that association of an odor with lc activation produces a learned preference for that odor.

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The Tastes of Polycose and Monosodium Glutamate in Humans. THOMAS P. HETTINGER, MARION E. FRANK and WALTER E. MYERS (University of Connecticut Health Center, Farmington, CT, USA)

If monosodium L-glutamate (L-MSG) has the "umami" taste (Kawamura & Kare, 1987) and Polycose, a mixture of glucose-containing oligosaccharides, has the "polysaccharide" taste (Sclafani, 1987), the existence of specific receptor systems for L-glutamate and Polycose has to be considered in molecular models of taste reception. Chemosensory profiles (Hettinger, Myers and Frank, 1990) for three concentrations (near-threshold to mid-range) of L-MSG, D-MSG, Polycose, maltose, sucrose, NaCl and $CaCl_2$ were based on choices of ten subjects, who tasted the substances with nose open or clamped. Subjects chose 1 or 2 of the following descriptors: sweet, salty, sour, bitter, metallic, sulfurous, soapy, other, and no-taste. 3.2% Polycose was primarily "sweet" with the nose open but tasteless when clamped. 10% and 32% Polycose were primarily sweet with nose open or clamped but "other" responses occurred for all concentrations with nose open. Polycose was the only substance affected by nose clamps, suggesting an olfactory component. At equimolar concentrations, the profiles for Polycose and maltose did not differ in the nose-clamped condition, but sucrose elicited more sweet responses. L-MSG and D-MSG had qualities that differed from the salty quality of NaCl and bitter quality of $CaCl_2$. 10mM L-MSG, 100mM D-MSG, 30mM NaCl, and 30mM $CaCl_2$ had similar perceptual salience. At these concentrations, L-MSG was bitter and had an "other" quality but D-MSG was salty, soapy and metallic. The "other" quality of the L-form, which has a low threshold, might be "umami". In conclusion, the idea of a "polysaccharide" taste in humans is not supported but there may be a chiral receptor site for "umami", which could be probed with analogs.

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The Taste of MSG Before and After Salt-Adaptation: Assessment Using Three Different Rating Tasks. ELAINE T. DEHAN, NICOLETTE J. VAN DER KLAAUW & ROBERT A. FRANK (University of Cincinnati).

Some researchers have proposed a separate taste system for the taste of monosodium glutamate (MSG), based on the observation that Asian subjects identify the taste of MSG as "umami", rather than using the four usual taste descriptors. Others have argued against this fifth taste, since subjects do not categorize the taste of MSG as "other" when given the opportunity, but rate MSG as partly salty, sweet, sour and bitter. We used three instructional sets to evaluate the taste of MSG, before and after salt-adaptation. Three groups of 15 subjects evaluated sucrose, NaCl, MSG, QHCl, citric acid, and distilled water. Group 1 rated saltiness only, Group 2 rated sweetness, saltiness and umami taste, whereas Group 3 rated sweet, salty, sour, bitter and other. Subjects were presented with four random blocks of the 6 stimuli, and rated two blocks after adaptation to water, and two blocks after adaptation to NaCl. When subjects rated saltiness only, the saltiness of MSG decreased significantly after salt-adaptation, though the saltiness of NaCl decreased to a greater extent. When subjects rated sweet, salty and umami, MSG was perceived as equally salty and umami tasting. After adaptation the saltiness of MSG significantly decreased, but its umami taste increased slightly. Thus, some distinct component of the taste of MSG persisted after adaptation. Finally, when subjects made 5 ratings, MSG was perceived as predominantly salty, and partly bitter, sweet and sour, but was not categorized as "other". After adaptation, the saltiness of MSG decreased, but the other qualities remained stable, with a slight increase in bitterness. The results from Group 1 and Group 3 could be used to argue that the taste of MSG and NaCl are coded in the same way. However, from the results of Group 2, it would be concluded that only part of the taste of MSG is coded through the salty pathway, because the perceived umami taste was unaffected by salt-adaptation.

6-n-propylthiouracil (PROP) Supertasters and Women Have Greater Number of Fungiform Papillae Taste Buds. VALERIE B. DUFFY[1], INGLIS J. MILLER, JR.[2], LINDA M. BARTOSHUK[1] (1. Yale University School of Medicine, New Haven, CT; 2. Bowman Gray School of Medicine, Winston-Salem, NC).

Our aim was to demonstrate that the tremendous variation in PROP tasting associates with the density of taste buds. Seventy-six subjects (ages 18 to 40), recruited by poster and word of mouth, participated in: PROP threshold; PROP and NaCl scaling; and videomicroscopic studies to identify a sample of fungiform papillae (FP), and taste pores (TP) on the right dorsal tongue tip. PROP and Anatomy Association. PROP tasting (threshold, ratio of PROP/NaCl) was regressed on the anatomical measures. Both PROP ratio and threshold showed a statistically significant relationship with the density of FP ($r=0.48$, $p<0.001$; $r=0.36$, $p<0.01$) and TP ($r=0.63$, $p<0.001$; $r=0.48$, $p<0.01$). However, PROP threshold only divides subjects into nontasters (NT) and tasters. PROP ratio further subdivides the tasters into medium tasters (MT) and supertasters (ST). When examining PROP tasters, only PROP ratio explained a significant amount of variance in TP density ($r=0.51$, $p<0.001$). A lower threshold in PROP tasters did not significantly associate with a higher TP density. Sex effects. Women ($n=40$) gave a higher average bitter rating to PROP than men ($n=37$) ($p=0.01$). In this sample, there was no significant difference in average PROP threshold between women and men. Women had higher average TP ($p<0.005$) and FP densities ($p=0.001$) than men. Sex by PROP effects. For two-way analysis of variance, PROP tasting was separated into non-taster (NT), medium taster (MT) and supertaster (ST). Sex and PROP status had significant main effects on TP density. Women had higher TP density than men ($p<0.05$) and ST had higher TP density than MT or NT ($p<0.0001$). In the interaction, women STs also tended to have more TP than men STs ($p=0.07$). Summary. These data continued to support our hypothesis that differences in ability to taste PROP strongly associate with the number of fungiform taste buds (Reedy, 1993). In addition, suprathreshold classification of PROP tasting explained more of the variance in fungiform taste buds than threshold classification. Higher ability to taste PROP in women is supported by a higher density of fungiform taste buds.

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Preliminary Examination of Suprathreshold Olfactory and Taste Perception: Free-Living Elderly Females Exhibit Greater Olfactory Than Taste Impairment. LAURIE A. LUCCHINA[1], LINDA M. BARTOSHUK[2], VALERIE B. DUFFY[2], ANN M. FERRIS[1], LAWRENCE E. MARKS[3]. (1. University of Connecticut, Storrs, CT; 2. Yale University, New Haven, CT; and 3. John B. Pierce Laboratory, New Haven, CT.).

Suprathreshold olfactory and taste function were assessed in 10 elderly (aged 65 years and older) and 10 young (aged 18-35 years) females using a new portable magnitude matching test. Elderly subjects were tested in their residences, and controls in the laboratory. Prior to each trial, the test was explained to the subject and practiced to ascertain comprehension of the procedure and the magnitude estimate scaling system. Perceived intensity ratings of randomized tastants [citric acid (range 0.001-.032M), sucrose (0.032-1M), quinine hydrochloride (0.000032-0.001M), and 6-n-propylthiouracil (PROP) (0.0001-0.0032)], and odorant [n-amyl acetate (1-10,000 ul)] were assigned. Data were normalized to sodium chloride taste (range 0.032-1.0M), low frequency noise (band width 100-500 Hz; 55-85 dB levels), and a 5-point adjective scale (very weak-very strong). Sodium chloride taste proved to be the best to normalize magnitude estimates, as perception of the low-frequency noise bands was evidently impaired for elderly subjects. Normalizing to adjectives yielded data similar to taste normalization. The results of this pilot study suggest that suprathreshold whole mouth taste function is generally stable with aging, although slight quality-specific losses may be exhibited. In contrast to taste, suprathreshold olfactory function was clearly impaired with aging: elderly subjects assigned lower perceived intensity ratings at all five odorant concentrations.

Supported in part by the University of Connecticut Research Foundation and NIH grant DC00283.

Correlation Patterns of Human Thresholds for Sucrose Octaacetate, Denatonium Benzoate, Quinine Sulfate and Propylthiouracil.
DAVID B. HARDER (Department of Psychology, Florida State University)

Thresholds for four bitter substances (SOA, DB, QSO4 and PROP) were obtained, via a sorting procedure, from 129 students enrolled at F.S.U.. Test-retest reliabilty data for SOA and DB were obtained from an additional 47 students. The unimodal SOA and QSO4 distributions were very similar (both medians= 3.7μM). The DB distribution was similar, but at much lower concentrations (median= 0.012μM). The PROP distribution spanned a wider range than the others (median= 37μM). No correlation was found between PROP thresholds and thresholds for the other three substances. The SOA, QSO4 and DB thresholds were positively intercorrelated. Partial correlation patterns suggested that the SOA-QSO4 covariation resulted from common effects of DB variation. High- and low-threshold groups for each substance were compared on the other three compounds. Results similar to the correlation patterns were found. The high and low PROP groups did not differ on any other substance. Nor did the other high and low groups differ on PROP. In contrast, the SOA, QSO4, and DB groups each differed on both other compounds too. The SOA and DB retest distributions closely resembled the original distributions. The individual test-retest correlations were low, however. Therefore, while SOA, QSO4 and DB thresholds covaried somewhat, the correlation coefficients indicated only lower limits on the extent of commonality. Similarly, while PROP sensitivity appeared distinctly less related, complete independence was not demonstrated.

Sensitivity to the Bitterness of Iso-α-acids: The Effects of Age and Interactions with NaCl. YOSHIKO YOKOMUKAI[1, 2], PAUL A.S. BRESLIN[1], BEVERLY J. COWART[1] & GARY K. BEAUCHAMP[1] ([1] Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA; [2] Kirin Brewery Co., Ltd. Tokyo, JAPAN).

We previously demonstrated an age-related loss of sensitivity to quinine sulfate (QS) but not urea (U) at both threshold and suprathreshold levels (Yokomukai, Cowart & Beauchamp, AChemS, 1993). In a sample of 45 young (< 40 years of age) and 48 elderly (> 65 years of age) subjects, we have now obtained measures of threshold sensitivity to tetra hydro iso-α-acids (T), the principal bitter compounds in beer. We found that thresholds for T were significantly higher in elderly subjects than they were in young adults. Subjects also rated the bitterness intensity of 5 suprathreshold concentrations of U (0.06-0.3M) and T (7-35ppm) using a 13-point category scale. Young subjects assigned significantly higher suprathreshold bitterness ratings to T than did the elderly. Thus, T showed greater overall similarity to QS than to U in that both threshold sensitivity and suprathreshold ratings were decreased with age. To further characterize the perceptual properties of T, its bitterness was rated in a separate experiment by magnitude estimation when presented in mixture with different concentrations of NaCl. We have shown that the bitterness of U and, to a lesser extent, QS is suppressed when presented in mixture with NaCl (Breslin & Beauchamp, AChemS, 1993). In the present study, we found the bitterness of T to be enhanced by mixture with NaCl. This is the first instance of bitterness enhancement with NaCl we have observed. The basis for this difference between T and other bitter compounds is not yet clear.

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Magnitude Matching and Scaling of Citric Acid on the Anterior Tongue in Humans

R. A. Englehardt (U. North Carolina)
J. Zuniga, N. Chen, C. Phillips (U. North Carolina)

The purpose of this study was to further investigate the relationship between fungiform taste bud density and taste sensitivity on the anterior tongue in humans. Citric acid solutions were delivered to spatially-matched flow chambers attached to the right and left sides of the anterior tongue of 60 normal volunteers in random order. A cross-modal magnitude matching procedure was used to scale taste intensity judgements for the right and left sides of the anterior tongue. The sip and spit method was used to contrast the spatial condition with whole mouth stimulation. Each estimate of taste intensity was adjusted by the subject's mean estimate of the brightness of a visual stimulus. The taste buds within the chamber were stained with methylene blue and recorded by videomicroscopy. Multivariate correlation analysis demonstrated a positive relationship between intercept ($p<.0001$, $r=.6$) and regression ($p<.0001$, $r=.51$) coefficient and the density of fungiform papillae and pores. The slope and intercept were significantly different in subjects with a high density of taste buds per chamber than in subjects with a low density ($p<.0001$). The power function was much greater under whole mouth conditions compared to spatial, implying greater sensitivity. Gender, age, race, and side had no significant effect on spatially-matched function. However, the combination of race and gender was associated with differences in slope intercept ($p=.01$). A larger sample size will be required to further evaluate this finding. In summary, our findings support evidence that variation in human taste sensitivity on the anterior tongue may be due, in part, to the numerical density of fungiform taste buds.

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Withdrawn

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Effect of Expectations on Hedonic Ratings and Taste Perception.

DEBRA A. ZELLNER (Shippensburg University)
SHAWN ROWE (Shippensburg University)
SORAYA CENTENO (Shippensburg University)

Hedonic ratings of flavors can be altered by changing expectations concerning how good they will taste, however, no study has yet demonstrated that taste perceptions can be similarly altered. In the present study we paired one color with a hedonically positive sweet taste while pairing a second color with a hedonically negative salty taste. Thus, the colors should result in expectations of either sweetness or saltiness. The sugar (sweet) and salty (NaCl) solutions contained the same four flavors, each presented three times. After sipping and spitting the color-taste pairs while giving hedonic ratings, subjects were given the four flavors four times without the tastants (sugar or salt) added. The flavored solutions did contain the salt-paired color half of the time and the sugar-paired color the other half of the time. Subjects rated how much they liked the taste of each sample and the saltiness and sweetness of each sample. Subjects rated the solutions containing the sweet-paired color as significantly sweeter than the same solutions containing the salt-paired color, $T(n=22)=46$, $p<.01$. There were no significant differences between the differently colored solutions on saltiness or liking ratings. The results provide some support for the idea that expectations can alter taste perceptions.

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Synergism Among Binary Mixtures of Fourteen Sweeteners.

SUSAN S. SCHIFFMAN (Duke University), ELIZABETH A. SATTELY-MILLER (Duke University), BREVICK G. GRAHAM (Duke University), SUZANNE D. PECORE (The NutraSweet Co.), BARBARA J. BOOTH (The NutraSweet Co.), B. THOMAS CARR (The NutraSweet Co.), and MICHAEL L. LOSEE (The NutraSweet Co.)

The purpose of the present study was to determine the degree of synergism among all binary mixtures of fourteen sweeteners varying in chemical structure. A trained panel evaluated binary combinations of the following sweeteners: 3 sugars (fructose, glucose, sucrose), 2 polyhydric alcohols (mannitol, sorbitol), 2 terpenoid glycosides (rebaudioside-A, stevioside), 2 dipeptide derivatives (alitame, aspartame), 1 sulfamate (sodium cyclamate), 1 protein (thaumatin), 2 *N*-sulfonyl amides (acesulfame-K, sodium saccharin), and 1 dihydrochalcone (neohesperidin dihydrochalcone). Each sweetener was tested at three concentrations which were isointense with 3%, 5%, and 7% sucrose. Panelists rated all fourteen sweeteners at each intensity level in mixtures with the same fourteen sweeteners at the identical intensity level. Both synergism and suppression of sweet taste intensity occurred depending upon the chemical classifications of the sweeteners in a combination. The greatest occurrence of synergism depended upon the intensity level of the combination. Synergism occurred most frequently in mixtures of sweeteners that were isointense with 3% or 5% sucrose. The mixtures with the greatest degree of synergism were those which included sugars, polyhydric alcohols, and the dihydrochalcone. Sweetness intensity ratings for the dipeptide derivatives, *N*-sulfonyl amides, and terpenoid glycosides tended to be suppressed when sweeteners within each group were mixed with themselves or with the other sweetener in the group. The greatest degree of synergism resulting from the combination of high potency sweeteners was with a mixture of thaumatin and neohesperidin dihydrochalcone at the concentrations equivalent with 3% sucrose.

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Cyclic AMP and cyclic GMP Mimic the Effect of Artificial Sweeteners in Isolated Hamster Taste Cells. THOMAS A. CUMMINGS, CHRISTI DANIELS, & SUE C. KINNAMON (Colorado State University and the Rocky Mountain Taste and Smell Center).

In previous studies we have shown that sweeteners elicit action potentials in a subset of hamster taste buds *in situ*, and that cAMP and cGMP elicit similar responses only in those buds that are sweet-responsive (Cummings & Kinnamon, *J. Neurophysiol.*, 70(6):522-534, 1993). We have also shown, in patch-clamp studies of isolated hamster taste cells, that sweeteners decrease a voltage-dependent K^+ current and that cAMP mimics the response (Cummings & Kinnamon, *Chem. Senses* 16:511, 1991). In order to induce the requisite depolarization for action potentials, the K^+ current blocked must be open at rest. In this study we have used giga-seal whole cell recording to examine the effects of sweeteners and cyclic nucleotides on leak conductances. Leak currents were monitored in response to bath applications of NC-000274-01 (NC01), 8cpt-cAMP, and dibutyryl-cGMP with intervening washes at a holding potential of -80 mV. To examine the effects on membrane conductance, hyperpolarizing voltage pulses were applied to the pipette at regular intervals. The bath contained elevated K^+ (20 mM), making E_K positive to the holding potential. In a subset of taste cells, the sweetener reduced the inward holding current and increased the input resistance. These results are consistent with a decrease in a resting K^+ current. Both 8cpt-cAMP and dibutyryl-cGMP mimicked the effect of the sweetener in sweet-responsive taste cells. In cells that did not respond to the sweetener, the nucleotides did not have an effect. These data support the hypothesis that in hamsters, the sweet response is mediated by cAMP or cGMP via closure of a resting K+ current to depolarize the cell to threshold.

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Paracellular Junction Potentials Contribute Quantitatively to Salt Taste Responses in the Hamster *Chorda Tympani*. HARRY WMS. HARPER (Duck Engineering Design, 500 E. 63rd St., New York, N.Y. 10021)

A salt taste transduction model has been proposed (the **Diffusion Potential Model**, *ISOT IX*) in which the nerve response is determined by two variable potentials: the resting potential of cation-selective taste cell receptor membranes; and a liquid junction potential arising at the paracellular junctions, where the different electrolyte solutions of the interstitial fluid and the taste pore contents (that is, the stimulus) come in contact. In this model, excitatory current flows in through receptor membranes, spreads through cell interiors and out through lateral cell membranes, and returns to receptor membranes by way of the interstitial fluid of the taste bud, the paracellular junctions, and the taste pore. This electrical geometry places the two variable potentials in series. The model predicts that the different responses to a fixed activity of a given cation, paired with different anions (the "anion effect"), will be a linear function of the liquid junction potentials. Previously (AChemS XIII), it was shown that this is true for 11 0.1 M Na salts, and noted that the slope of this function, which gives the voltage sensitivity of the response, is an important parameter for any electrogenic salt transduction model. More generally, the model predicts that the difference in responses to sodium salts at *any* Na activity will be given by the difference in liquid junction potentials, scaled by the voltage sensitivity of the response. Here it is reported that this prediction is quantitatively confirmed for responses to NaCl and Na_2SO_4, throughout their entire concentration-response ranges. There are no free parameters in this test of the **Diffusion Potential Model**.

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Rat Tongue Epithelium Has Basolateral Amiloride-Sensitive Na^+-Transport Pathway. SHEELLA MIERSON, MICHELLE MARIE OLSON, AMY TIETZ, TERRI MACHTINGER, BARBARA K. GIZA, AND THOMAS R. SCOTT (University of Delaware, Newark, DE 19716.)

Previous authors (Q.Ye, G.L.Heck, & J.A.DeSimone, *J.Neurophysiol.*, 1993, *70*:167-178; B.R.Rehnberg, B.I.MacKinnon, T.P.Hettinger, & M.E.Frank, 1993, *J.Gen.Physiol.*, *101*:453-465) have proposed a model of taste cell function in which some taste cells have an amiloride-sensitive Na^+ channel in the basolateral membrane. The model would account for the portion of the neural NaCl taste response that is insensitive to mucosal amiloride; some Na^+ would diffuse across the tight junction and into the taste cell via this basal pathway. To test this model we measured ion transport properties of the *in vitro* rat tongue epithelium under voltage clamp in an Ussing chamber. To ensure accessibility to the basolateral membrane, connective tissue was removed by injecting pronase solution under the epithelium. Short-circuit current (I_{sc}) was reduced by amiloride in the submucosal solution. The pattern of sensitivity of I_{sc} to submucosal amiloride differed in several respects from the pattern for mucosal amiloride: (1) The percent inhibition was greater. (2) The K_i was higher, approximately 50 μM amiloride. (3) The selectivity for Na^+ over K^+ was less; with 0.5 M NaCl or KCl, respectively, in the mucosal side, the ratio of inhibition of I_{sc} was approximately 2:1. As the concentration of mucosal NaCl was varied, there was little inhibition of I_{sc} below isosmotic concentration due to submucosal amiloride; the % inhibition increased as mucosal salt concentration increased. With 0.5 M sodium gluconate in the mucosal solution, submucosal amiloride had little effect. These last two findings agree with predictions based on the model of Ye *et al.* Preliminary results using multi-unit recordings from the *nucleus tractus solitarius* indicate that the taste response to a variety of stimuli is diminished when amiloride is perfused through the lingual artery.

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Voltage-Dependent Calcium Currents in *Necturus* Taste Receptor Cells Are Modulated by Serotonin Via Two Different Second Messenger Pathways DELAY R.J., S.C. KINNAMON and S. D. ROPER (Dept. of Anatomy & Neurobiology, Colo St Univ. and the Rocky Mt. Taste & Smell Center, Univ. Colo Health Science Cntr)

Cells in the taste buds of *Necturus* can be divided into receptor cells (cells which extend to the taste pore) and basal cells (cells located at the base of the taste bud). Recent studies (Delay, Taylor and Roper, 1993) have shown that Merkel-like basal cells (a subset of basal cells) contain serotonin (5-HT) and may release 5-HT in response to taste stimulation. In addition, Merkel-like basal cells form chemical synapses with receptor cells and with nerve fibers in *Necturus* taste buds (Delay and Roper, 1988). Thus, Merkel-like basal cells are in a position to act as interneurons within the taste bud, modulating the response of the taste receptor cells. In this study we have used giga-seal whole cell recording to examine the effect of 5-HT on voltage-activated Ca^{2+} currents (I_{Ca}) in isolated taste receptor cells. Two different effects of 5-HT were observed. Approximately 33% of receptor cells responded to 100 μM 5-HT with an *increase* in peak I_{Ca} (mean increase = 55%). The effect was reversible. Serotonin increased Ca^{2+} currents in responsive cells at concentrations as low as 1 μM. The increase in I_{Ca} was mimicked by 8cpt-cAMP (2 mM), blocked by H-89 (a cAMP-dependent kinase inhibitor) and insensitive to pertussis toxin (PTX). In a second subset of taste cells (67%), 100 μM 5-HT caused a reversible *decrease* in the peak I_{Ca} (mean decrease = 45%). This effect was PTX-sensitive. The decrease in I_{Ca} by 5-HT could be abolished by treatment with H-7, a non-selective protein kinase inhibitor.Thus, 5-HT modulates I_{Ca} of taste cells by two different 5-HT receptors that are linked to two different second messenger pathways. The results suggest that a G-protein-activated, cAMP-dependent protein kinase is responsible for the potentiation of I_{Ca} by 5-HT. The reduction of I_{Ca} by 5-HT appears to be mediated by a PTX-sensitive G-protein through activation of a protein kinase. Preliminary experiments suggest this kinase is neither PKA or PKC. These results suggest that 5-HT modulates transmitter release during taste stimulation thus implying a neuromodulatory role for serotonergic Merkel-like basal cells.

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Amiloride-Sensitive Na^+ Currents in Taste Cells Isolated from Neonatal Rats. MARTHA MCPHEETERS[1,3], JOHN C. KINNAMON[2,3]& SUE C. KINNAMON[1,3]. ([1]Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523, [2]Department of Biological Sciences, University of Denver, Denver, CO 80208, [3]Rocky Mountain Taste and Smell Center, Denver, CO 80262.)

Amiloride-sensitive Na^+ channels have been shown to be largely responsible for NaCl taste transduction. Immunoreactivity to the amiloride-sensitive Na^+ channel has been detected in taste cells of postnatal rats from Day 1 onwards (Stewart & Hill, 1993, In: Mechanisms of Taste Transduction, eds. Simon & Roper,127-158), yet amiloride is ineffective in blocking the chorda tympani response to NaCl until Day 12-13 (Hill & Bour, 1985, Dev. Brain Res. 20, 310). To resolve this apparent discrepancy, we have used giga-seal whole-cell recording to examine isolated taste buds from postnatal rats for the presence of amiloride-sensitive Na^+ currents. Individual taste cells were voltage-clamped at -80 mV in an extracellular solution containing 140 mM NaCl and holding current was monitored in response to bath application of amiloride (30 μM). In taste cells from early neonates (Day 2-13), amiloride decreased the inward holding current and increased the input resistance in 39% of the taste cells tested (n=18). The effects of amiloride were mimicked by replacement of Na^+ with N-methyl-D-glucamine ($NMDG^+$), suggesting that amiloride was inhibiting an influx of Na^+. Amiloride-sensitive currents were observed in 2 day old rats, suggesting that functional amiloride-sensitive Na^+ channels are present at this time. In taste cells from older rats (Day 15-39), 36% of the taste cells (n=22) exhibited amiloride-sensitive Na^+ currents. These data provide evidence that the amiloride-sensitive Na^+ channels observed immunocytochemically may be functional channels and that other factors must account for the lack of amiloride sensitivity observed in whole nerve recordings.

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The Rat CT-Response to KCl is Insensitive to Field Voltage Perturbations: Implications for the Location of K^+ Transducer Sites. QING YE, GERARD L. HECK, JOHN A. DeSIMONE (Department of Physiology, Virginia Commonwealth University, Richmond VA, 23298-0551)

Chorda tympani (CT) recordings were made from rat tongue under epithelial voltage clamp (VC) using NaCl, KCl, and potassium gluconate (KGlu) as stimuli. As previously shown, responses to NaCl were enhanced at negative VC relative to the current clamp (CC) response and suppressed at positive VC. In contrast, responses to KCl were insensitive to field voltage perturbations. Responses to K^+-salts showed greater sensitivity to anions than responses to Na^+-salts. In CC mode, responses to 0.5 M KGlu were only about 14% of those to 0.5 M KCl. In addition the temporal structure of the CT responses between KCl and KGlu differed significantly. At 0.5 M the KGlu response onset required 12 sec to reach half maximal; for KCl the half-maximal time was 0.4 sec. Responses to KGlu showed a large off-response; those to KCl did not. The KGlu off-response coincided with the collapse of a hyperpolarizing field potential more than twice that of KCl, suggesting that the KGlu off-response resulted from the collapse of its own field potential. Measurements of transepithelial conductances and independent measurements of times to half-maximal CT response showed that the effective permeability coefficient for KCl diffusion in the paracellular pathway was 28 times greater than that for KGlu. This, and the voltage insensitivity of KCl responses, indicates that access to K^+ transduction sites is significantly diffusion-controlled. This accounts for the 15-fold increase in the apparent K_m in the dose-response relation for KGlu (4681 mM) relative to that for KCl (292 mM). The diffusion-free K_m for K^+ ion stimulation was estimated at 134 mM, which is not significantly different from the K_m (129 mM) of the amiloride-insensitive NaCl response. The results indicate that basolateral sites are responsible for most of the K^+ response and part of the NaCl response.

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The Fine-Scale Structure of Chemical Signals Within the Feeding Current of a Calanoid Copepod

PAUL A MOORE (Monell Chemical Senses Center), DAVID M FIELDS and JEANNETTE YEN (Marine Sciences Research Center, State University of New York

Chemical signals are thought to play an important role in the selection of particles during feeding in many copepod species. In addition, chemical cues may also play a role in the avoidance of predators and the selection of mates. Before these various behaviors can be studied in detail, it is essential to understand the temporal and spatial dynamics of chemical signals in the small scale local environment of the copepod, *i.e.* within the feeding current. Small scale (≈30 μm) chemical measures can be performed *in situ* using recent advances in chemical detection technology. This new technology (IVEC-5) can record chemical concentrations at rates up to 200 Hz using electrochemical techniques. We performed chemical measurements while simultaneously video taping particle movement within the feeding current of a marine calanoid copepod. Chemical signals measured at these scales without the presence of the copepod are typically diffusion-like in structure. Our results show that a small sphere of odor, such as that surrounding an algal particle, will be deformed greatly by the feeding current of the copepod. Odor patches will be stretched and sheared by the fluid forces within the feeding current. The resulting chemical signal is much more transient, much less variable and, in some locations near the animal, much stronger in intensity. The implications of these results for the detection of food particles by chemoreception will be discussed.

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Stimulus integration time of lobster olfactory receptor neurons. GEORGE GOMEZ and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole MA 02543).

Investigations of the dispersal of chemical stimuli in the environment have shown that odors are often distributed in discrete patches. Chemoreceptor cells perceive these patches as pulses of odor occurring over time. In addition, some animals such as lobsters take discrete intermittent samples of odor by antennule flicking, which further adds to the pulsatile nature of chemical stimuli. Previous studies have shown that lobster chemoreceptors rapidly adapt and disadapt to stimulus pulses; these adaptation and disadaptation properties may serve a fundamental role in lobster orientation. This study attempts to determine the limits of intrapulse adaptation by focusing on the stimulus integration time of lobster olfactory receptor neurons. Lateral antennules of intermolt lobsters were excised and situated in an open-faced olfactometer. Aesthetasc sensilla bearing hydroxyproline-sensitive cells were identified and localized with a concentric pipette focal stimulation system. Stimulus applications were monitored on-line with the In Vivo Electrochemistry Computer System (IVEC-5), which measured a tracer (dopamine) mixed in with the stimulus solution. On-line stimulus measurement allowed us to accurately quantify each stimulus presentation and calculate molecular delivery rates to the chemoreceptor cells. Receptor cells were stimulated with 1, 5, 10, 50 and 100 μM hydroxyproline steps of 50, 100, 200, 500, and 1000 ms in duration. Extracellular recording was used to evaluate receptor cell responses. At stimulus concentrations at or close to threshold, receptor cells responded with a few (1-3) spikes at latencies of 68 to 575 ms. Increased stimulus concentrations resulted in shorter first spike latencies (46 to 350 ms at 100 μM Hyp), increased spike numbers and increased instantaneous spike frequency. Cell responses increased both with increased stimulus concentration and duration; however, stimulus volume delivered over a shorter time period (i.e., short step at a high concentration) resulted in a greater cell response (e.g., increased spike frequency) than that same volume delivered over a longer time period (i.e., longer step at a lower concentration). This effect was evident for step durations of 100-200 ms in about half of the receptor cells. For these cells, odor steps longer than 200 ms did not result in a significant increase in cell response, indicating that this was their approximate stimulus integration time. Lower stimulus concentrations generally resulted in longer integration times.

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Turbulent odor plumes and chemo-orientation in nature.

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Orientation by animals using chemical cues often takes place in flow, where stimulus properties of odorants are affected by characteristics of fluid motion. Except for insects, past investigations have seldom linked either the success or the mechanism of odor-mediated search with the natural hydraulic environment in which it occurs. To examine the interaction between fluid dynamics and chemoreception, we investigated predatory search by blue crabs in odor plumes of attractants released from clam prey. We performed field experiments to: (1) construct computational models of odor plumes, and (2) analyze kinematics of crab locomotory responses to odor plumes. Mean flow velocities and turbulent mixing were measured in the benthic boundary layer; odor application and fluxes were scaled to mimic releases by natural prey; high speed electrochemistry was used to directly measure fine-scale concentration distributions; frame-by-frame video analysis was used to measure dye plumes and crab locomotion. Crab movements within plumes were oriented directly upstream, whereas passage outside of the plume boundary stimulated an immediate (within 600 ms) turn back into the plume. Flow and chemical information interact to permit efficient orientation. Perception of a chemical cue causes upstream locomotion; loss of the cue causes movement lateral to flow direction. Similarities and differences in the fluid dynamics between the cases of insects in air and crabs in water result in distinct odor plume structures and affect their perception. Comparative examination of boundary layer fluid dynamics yields insights that can explain mechanisms of orientation to odor plumes in these cases, and may have wide application to other organisms.

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Reiterative responses to single strands of odor promote sustained upwind flight and odor source location by moths

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We characterized single upwind surges of flying male *Heliothis virescens* moths in response to individual strands of pheromone generated experimentally in a wind tunnel. We then showed how this surge functions in this species as a basic 25-cm, 0.35-sec-long building block that is strung together repeatedly during typical male upwind flight in a normal pheromone plume. The template for a single iteration, complete with cross-wind casting both before and after the straighter upwind surging portion, was exhibited by males flying upwind to pheromone and experiencing filament contacts just frequently enough to produce successful upwind flight to the source, as hypothesized by an earlier model. Also as predicted, when filament contact by males became more frequent, only the straightest upwind portions of the surges were reiterated, producing direct upwind flight with little cross-wind casting. In-flight electroantennogram recordings (EAGs) made from males in free-flight upwind in a pheromone plume from a normal point source further support the idea that a high frequency of filaments encountered under the usual pheromone plume conditions promote only these repeated straight surges. In-flight EAGs also showed that when filament contacts cease, the casting, counterturning program begins to be expressed after a latency period of 0.35 sec. Taken together these results provide a plausible explanation for how male and female moths, and perhaps many other insects, fly successfully upwind in an odor plume and locate the source of the odor, using a surging-casting, phasic-tonic response to the onset and disappearance of each odor strand.

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Zonal Patterns of Odorant Receptor Gene Expression

LINDA B. BUCK, SUSAN L. SULLIVAN, and KERRY R. RESSLER
(Harvard Medical School, Boston, MA)

We previously identified a large multigene family that codes for between 500 and 1000 different types of odorant receptors which are expressed on mammalian olfactory sensory neurons. The unusual size and diversity of this family presumably reflect its ability to recognize a vast array of structurally diverse odorous ligands. We have begun to ask how the olfactory system might organize the information provided by such an enormous variety of receptors. We first examined the patterns of expression of different odorant receptor genes in the olfactory epithelium to ascertain whether there might be spatial maps for odors in the nose which could serve to organize sensory information. Our studies demonstrate that the mouse olfactory epithelium is divided into a limited number of 'expression zones' in each of which only a subset of the olfactory multigene family is expressed. In a single zone, only certain odorant receptor genes can be expressed, but many different receptor genes are expressed in that zone and neurons that express a particular gene are scattered throughout the zone. The zones are highly specified: they exhibit bilateral symmetry in the two nasal cavities and are virtually identical in different individuals. The zonal patterning that we have observed suggests that incoming sensory information may undergo an initial broad organization in the nose prior to its transmission to the brain. We have noted a striking correspondence between the odorant receptor expression zones and patterning of the axonal projection from the olfactory epithelium to the olfactory bulb as described by previous retrograde labeling and immunohistochemical studies. This correspondence suggests that the zonal organization observed in the epithelium is maintained in the axonal projection to the bulb.

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Rhinotopy as an Organizing Principle in the Olfactory System. THOMAS A. SCHOENFELD (Depts. of Psychology and Biology and the Neuroscience Program, Clark University, Worcester, MA 01610)

The projections of olfactory receptor neurons to the main olfactory bulb (MOB) are organized spatially according to their 3-dimensional position within the nasal cavity, rather than their 2-dimensional position along the mucosal surface (Schoenfeld et al., *Brain Res. Bull.*, in press; Clancy et al., *Brain Res. Bull.*, in press). We refer to this principle of organization as rhinotopy. Through rhinotopically organized projections, cavity airspace is represented to the MOB as longitudinally-oriented channels. For example, olfactory mucosal segments that line distinct medial and lateral channels project to the medial and lateral halves of the MOB, respectively, even though the segments may reside on the same turbinate. Projections to the two dorsal quadrants of the MOB originate from mucosal segments that line two relatively smooth, centrally-positioned channels anchored to the dorsal meatus (recess). On the other hand, projections to the two ventral MOB quadrants arise from segments that, within the caudal recesses, line relatively convoluted channels lying peripheral (ventral, lateral and even dorsal) to the dorsally-projecting central channels. Since the ventrally-projecting mucosa has almost three times the surface area of the dorsally-projecting mucosa, the rhinotopic map gives the smooth channels lined by dorsally-projecting segments a disproportionately larger representation on their half of the MOB than it does to the convoluted channels lined by ventrally-projecting mucosal segments. As in the receptotopic maps of other sensory systems, this disproportion may be used to establish labeled lines to the primary sensory cortex, i.e., the MOB, with enhanced resolving power or gain setting. Within the brain, the central circuits of the olfactory system are organized with divergent degrees of rhinotopic precision. An intrabulbar associational system, an interbulbar commissural system and bulbofugal projections to higher-order olfactory cortex exhibit greater, comparable or little to no rhinotopic precision, respectively, as compared to the precision of the primary afferents (Schoenfeld et al., *J. Comp. Neurol.*, 1985). Here, too, such hodological attributes may be used as they are in other receptotopically mapped sensory systems, to support a range of processing streams, from enhanced isolation of spatially-defined sensory features at early stages of analysis, particularly within and between the two MOBs, to complex, potentially space-neutral recombinations of features derived from common perceptual objects at later stages of processing within the piriform cortex and beyond.

Odorant Coding in the Olfactory System: Lessons from the Salamander. J. S. KAUER, A. R. CINELLI, K. DORRIES, A. JESURUM, J. WHITE. (Tufts Medical School and New England Medical Center, Boston, MA)

The air-breathing phase of the salamander, *Ambystoma tigrinum*, has served as a useful experimental animal for investigating mechanisms by which odorants are encoded in the vertebrate olfactory system. Among the advantages this preparation provides are: an anatomically simple and accessible olfactory epithelium (OE), relatively large olfactory receptor neurons (ORNs), planar olfactory bulb (OB) laminae, odor-guided behavior, robust slice preparations, large voltage-sensitive dye (VSD) signals, extensive electrophysiological data on the OE and OB, and, most recently, biochemical and molecular biological information on transduction mechanisms. A number of studies from various laboratories that have used this animal have focussed on how spatial and temporal patterns of response may contribute to the encoding of odorant properties. Results from single ORN cell recordings, EOG mapping, measurements of mitral/tufted (M/T) cell receptive fields (rfs), VSD recordings from the OE and OB, and behavioral experiments will be presented to illustrate principles, observed in this animal, that may relate to how odorants are represented by the nervous system.

Some of the major findings from these many studies are: single ORNs have broad spectrum responses; responsivity to single compounds is widely distributed across the mucosa; rfs for excitatory M/T cell responses are more restricted than for inhibitory responses; there is anatomical convergence and divergence of ORNs onto OB glomeruli; candidate molecular odorant receptors are found scattered in localized OE regions; there are differences in odor-guided behavior to various members in a series of homologous compounds. Based on these data an hypothesis of how odorants may be encoded by the salamander olfactory system will be presented.

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Odorant-Specific Spatial Patterns in Mucosal Activity: Correlation with Behavior. Steven L. Youngentob, Paul F. Kent and Paul R. Sheehe. (Clinical Olfactory Research Center, SUNY HSC, Syracuse, NY 13210).

There is considerable evidence, based upon a number of physiologic techniques, that different odorants produce different patterns of neural activity at the mucosal level. One apparent mechanism for this spatial differentiation of odorants is the regional variation across the olfactory mucosa in the sensitivity of the receptors to different odorants. One goal of olfactory physiology is to evaluate whether there exists a relationship between a candidate coding mechanism and the perception of the animal. Therefore, the present study examined the relationship between odorant-induced "inherent" activity patterns and odorant quality identification in the rat. Using operant techniques and an odorant confusion matrix task, Long-Evans Hooded rats were trained to differentially report (i.e., identify) the odorants propanol, carvone, citral, propyl acetate, and ethylacetoacetate. Following training, each animal was tested using a standard 5x5 confusion matrix design. The results of the behavioral tests were subjected to an MDS analysis which established a two-dimensional perceptual odorant space for each of the individual animals. At the completion of testing, each animal was sacrificed and their mucosal activity patterns were recorded using a voltage-sensitive dye technique. Using the dye, di-4-ANEPPS, we monitored the fluorescence changes at 100 contiguous sites in a 10x10 photodiode array on the olfactory mucosa of each rat's septum and turbinate in response to the same five odorants. For each animal, the ability of the relative position of the five electrophysiologically determined "hot spots", in two-dimensions, to predict their relative position in the two-dimensional odorant space was evaluated. The results of this analysis indicated a significant ability for the electrophysiological response to predict the position of the odorants in a perceptual space ($p<.02$). Thus, these data suggest for the first time, that mucosal "inherent" activity patterns serve as the substrate for the perception of odorant quality. (NIH Grants DC00220 &DC00072

Is Olfactory Space Irrelevant?
Burton M. Slotnick (Department of Psychology, The American University, Washington, DC 20016)

Continuing evidence from neuroanatomical and physiological studies indicate that spatial organization plays a significant or even central role in olfactory coding. However, behavioral studies provide little or no support for this view. The negative evidence includes:

1. Failure of rats with extensive lesions of 2-DG identified loci of the olfactory bulbs to show deficits in discrimination, detection, sensitivity or ability to recognize the odor used to produce the 2-DG loci.
2. Unilaterally bulbectomized rats with 30 - 80% of the remaining bulb removed performed nearly as well as controls on a variety of odor detection and discrimination tasks. Behavioral deficits were weakly related to size of lesion but no relationship was found between locus of the lesion and behavioral deficits.
3. As revealed by anterograde transport of HRP, the 'olfactotoxin' 3-methyl indole produces a loss of almost all peripheral afferents to most areas of the bulb. Treated rats had a general olfactory deficit but were able to detect each odor tested and there was no evidence for a specific anosmia despite the fact that glomerular areas identified in prior 2-DG studies (using the odors employed in these behavioral tests) had little or no functional input.

In general the results of these and related studies reveal considerable equipotentiality of function at the level of the olfactory bulb and suggest that neither localization demonstrated by metabolic studies nor a pattern of projections from the periphery to the bulb may form the basis for odor identification.

Not Available

The use of retroviral vectors to analyze cell lineage, proliferation, determination and migration during the development of the mammalian forebrain. MARLA B. LUSKIN (Dept. of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

Retroviral-mediated gene transfer has been extremely fruitful over the past ten years in furthering our understanding, at the cellular and molecular level, of the means by which the mammalian forebrain develops. This technique utilizes replication defective recombinant retroviruses as vectors which are capable of introducing foreign genes into dividing cells. Most significantly, retroviral-mediated gene transfer has made it possible to introduce a heritable marker into individual progenitor cells or a defined population of progenitor cells in the intact mammalian central nervous system at various times during pre- and postnatal development, which heretofore has not been possible. At some later time point, the position in the brain and phenotype of the descendants of virally infected cells can be analyzed. For such purposes the most commonly used retroviral lineage tracers have had the reporter gene *E. coli* ß-galactosidase, whose protein product is abbreviated lacZ, added to their genome. The expression of the lacZ protein can be detected histochemically or immunohistochemically at the light and electron microscopic level in individual cells. We have used retroviral-mediated gene transfer to determine how cell diversity arises in the forebrain and to track the proliferation and migration of populations of neurons destined for the olfactory bulb. An analysis of the lineage relationships of among cells of the cerebral cortex has demonstrated that by the onset of cortical neurogenesis, that there are separate progenitor cells for neurons, astrocytes and oligodendrocytes in the ventricular and subventricular zones, where cell proliferation occurs. In addition, we have shown that there is a specialized region of the postnatal subventricular zone containing exclusively neuronal progenitor cells, whose progeny are capable of undergoing cell division en route to the olfactory bulb, where they differentiate into granule and periglomerular cells.

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Retroviral lineage studies of the rat olfactory epithelium. J.E. SCHWOB*, J.M.T. HUARD*, M.B. LUSKIN§, S.L. YOUNGENTOB†. (* Dept. Anat. and Cell Biol. and *,† Clinical Olfactory Res. Ctr., SUNY Health Sci. Ctr., Syracuse, NY 13210 and §Dept of Anatomy and Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The olfactory epithelium (OE) of mammals has the unique property of generating sensory neurons (OSN's) throughout life. However, neither the precursor populations responsible for neurogensis and epithelial reconstitution nor the lineage relationships among the various cell types have been definitively identified. To characterize these processes, we have undertaken lineage studies of the rat OE using three different replication incompetent retroviral vectors containing a reporter gene. The three vectors are the BAG, MMuLV-SVnlsLacZ, and DAP vectors. The first two of these encode beta-galactosidase as the marker enzyme but the enzyme is localized to the nucleus of the second, while the third encodes alkaline phosphatase; thus infection by the different vectors in the same animal can be distinguished by the type of marker enzyme and its intracellular distribution. Retrovirus vector (RV) was delivered by direct injection into the OE of normal adult rats or by infusion into the nasal cavity of rats lesioned by inhalation of MeBr. After staining the epithelium for the marker enzyme as a whole mount, the phenotype of the labeled cells was determined by immunochemistry on tissue sections. As a result of direct injections, clusters of labeled cells were centered on the injection site and were composed of neurons and globose basal cells. However, we cannot say with any certainty that a cluster of infected cells is derived from a single founder cell. After MeBr lesion, the clusters of virally-marked cells are separated by a mm or more, suggesting that each cluster consists of cells derived from a single founder cell and that each, therefore, is clonal in nature. We are directly verifying the identification of clusters as clones by exposing the lesioned epithelium to mixtures of the vectors; homogeneity in terms of vector expression strongly suggests that all the cells of a cluster derive from a single founder cell. By 12 days after MeBr lesion the clusters can exceed 100 cells in number and can include both neuronal and nonneuronal cells in the same cluster; in some cases the nonneuronal cells extend below the basal lamina. Smaller clusters, on average, tend to contain only a single cell type whether neuronal or supporting cell. At survivals of 21 and 30 days the clones are smaller on average. The results suggest that the intact OE is characterized by the progression of globose basal cells to neurons as previously suggested. However, our results suggest that during recovery from epithelial injury a progenitor may ultimately give rise to both neuronal and nonneuronal cells.

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Analysis of Olfactory Epithelial Cell Lineages Using a Replication Incompetent Retrovirus. MARY E. CAGGIANO, JOHN S. KAUER, DALE D. HUNTER (Neuroscience Program. Tufts/New England Medical Center, Boston, MA. 02111)

It has long been known that vertebrate olfactory receptor neurons (ORNs) have the unusual properties of a short life-time and the ability for self-renewal. There is good evidence that new neurons arise from cells in the basal regions of the mucosa. Presumptive progenitor cells in these basal regions are broadly characterized into two populations (horizontal and globose basal cells); these two populations respond differentially to removal of their target the olfactory bulb, a procedure which causes degeneration of ORNs. With ablation of the bulb, globose cells show enhanced division and new ORNs are generated, partially restoring mucosal structure. The precise relation between the neurons (and/or other cells in the epithelium) and basal cells is, however, not yet fully defined. We have examined this developmental process by labeling progenitor cells and their progeny using a replication incompetent retrovirus (DAP) that carries the gene for a histological marker (alkaline phosphatase).

DAP virus was injected through the skull into the region of the olfactory epithelial septum in 7-10 day old rats; some animals had unilateral olfactory bulbectomies 5 days before infection. Regions of the septum were examined at 5, 20, and 40 days after infection by analyzing whole mount preparations for incorporation of the retrovirus into cells within the epithelium. Areas showing alkaline phosphatase activity were then cut in transverse section. Under these conditions, scattered groups of small, rounded cells with darkly stained cytoplasm and unstained nuclei were seen in anterior and posterior septal regions. Cells were identified by their position in the epithelial layers and by immunohistochemical staining with OMP, NCAM, and cytokeratins.

This study provides evidence that globose basal cells give rise to new ORNs, that horizontal basal cells do not appear to give rise to globose cells, and that there is another, separate lineage for sustentacular cells.

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Amphibian taste buds arise from endoderm. LINDA A. BARLOW and R. GLENN NORTHCUTT (University of California, San Diego).

Taste buds are known to differentiate within the oral epithelium of vertebrate embryos, however, the embryonic source of these cells has not been ascertained. Three possibilities were explored: 1. neural crest cells; 2. cells of the epibranchial placodes, which give rise to portions of the sensory ganglia of the branchiomeric cranial nerves; and 3. endodermal cells, which line the oral cavity of ambystomid salamanders. To test the possibility that taste bud precursor cells arise from neural crest or epibranchial placodes and migrate into the oral epithelium, we transplanted the appropriate region from a pigmented embryo into an albino host. We then examined the distribution of pigment granules in these animals shortly after hatching. In all cases, pigmented taste buds were never encountered, whereas the expected derivatives of neural crest and placodes contained numerous pigment granules. To demonstrate taste buds arise directly from the endodermal epithelium lining the mouth, we microinjected DiI, a carbocyanine dye, into the presumptive endoderm at the onset of gastrulation, and then allowed the embryos to develop to hatching. The oral epithelia of these animals contained clusters of DiI-labeled cells composed of both generalized epithelial cells and taste buds. We conclude from these studies that taste buds in axolotls arise solely from endoderm.

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Use of Chimeric Mice in the Analysis of Taste Bud Cell Lineage. T. FINGER, L.M. Stone (Rocky Mountain Taste & Smell Ctr., Univ. Colorado Sch. Medicine, Denver, CO).

Interactions between gustatory nerves and epithelial cells are important in the generation of taste buds (Mbiene & Farbman, 1991). Unclear however is whether the epithelial progenitor cells that give rise to the taste receptor cells are derived from local lingual epithelium or whether the progenitor cells migrate in from neurogenic tissues such as neural crest or epibranchial placode. Also unknown is whether each taste bud arises from a single progenitor or from multiple progenitor cells. One way to address these questions is through analysis of tissue from animals consisting of 2 genetically distinct populations of cells. Two types of such mice were utilized in these studies: 1) aggregation chimerae formed by the fusion of early morulae from two different strains (one carrying a globin transgene marker), and 2) X-inactivation mosaic mice in which one X chromosome carries a transgenic lac-Z marker. The tissues of chimeric or mosaic animals are a quiltwork of patches of cells derived from one or the other lineage. If taste buds form as progeny of migratory neurogenic cells, then the phenotype of the taste receptor cells will not always match that of the surrounding epithelium. Similarly, if the cells of each taste bud originate from a single progenitor then the phenotype of cells within each taste bud always will be uniform. Our studies show that the phenotype of the cells within each taste bud always matches that of the surrounding epithelium. Further, for taste buds situated at the border of two epithelial patches containing cells of the different lineages, the taste buds may contain cells of each lineage. These results indicate that taste buds arise from multiple progenitors and that these progenitors derive from local epithelium, not from migratory neurogenic populations. This talk will emphasize methods, data analysis and pitfalls rather than the results which are presented separately (see Stone et al., this meeting).

Effects of Stimulus Novelty on NaCl Taste-Induced Expression of C-*fos* in the Central Nervous System of Golden Hamsters. MICHAEL A. BARRY, ELISSA J. CHESLER, and DAVID C. LARSON (Department of BioStructure and Function, University of Connecticut Health Center).

We are using the expression of the immediate early gene, c-*fos*, as a marker for neuronal activity and protein synthesis induced by gustatory stimulation. It has been shown that protein synthesis in the insular cortex is necessary for the formation of memory for taste stimulus familiarity (Rosenblum et al., Behav. Neural Biol. 59:49,'93). The processing of novel stimuli may be associated with an increase in protein synthesis and immediate early gene expression in cortex. Alternatively, a formed memory for familiar stimuli may affect the processing of gustatory stimuli and c-*fos* expression in brainstem gustatory nuclei.

We first showed that a series of presentations of 0.1 M sucrose or 0.15 M NaCl would result in significant stimulus familiarity in golden hamsters by utilizing the phenomenon of learned harmlessness in a conditioned taste aversion (CTA) paradigm. Hamsters with preexposure to the unconditioned stimulus showed a significantly reduced aversion to the stimulus following a CTA relative to those preexposed to water. The phenomenon of learned harmlessness had not been previously demonstrated in hamsters.

Water deprived hamsters were given a drink of 0.15 M NaCl. After two hours the animals were sacrificed, and their brains were processed to reveal c-*fos* protein with immunohistochemistry. Ten hamsters had a prior series of presentations of NaCl (familiar group), and ten were preexposed to water (novel group). Preliminary results indicate that there was greater *fos* expression in the novel than the familiar group in insular cortex but not in the solitary or parabrachial nuclei.

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Fos labeling in the parabrachial nucleus after electrical stimulation of taste nerves in lightly-anesthetized rats. THERESA A. HARRISON and NANCY W. MILLER (Cellular Biology & Anatomy, Medical College of Georgia)*

In previous studies, we were able to immunocytochemically demonstrate Fos protein distribution within the nucleus of the solitary tract following electrical stimulation of taste afferent nerves in deeply anesthetized rats, but were unable to detect Fos labeling at successive levels within the taste pathways. In the present study, we report that stimulus-specific Fos labeling can be seen in the parabrachial nucleus (PBN) following peripheral nerve stimulation when rats are maintained in a lightly anesthetized, rather than deeply anesthetized, state during the period of stimulation. Following surgery to expose either the chorda tympani nerve (VII), or the lingual-tonsilar branch of the IXth nerve, rats were maintained under Nembutal anesthesia such that strong pinch of the forepaw produced a slight withdrawal response. After 2 to 3 hours of stimulation with 500 msec trains of pulses (200 µsec duration) at 83 Hz applied 1/sec, Fos-positive neurons were seen ipsilaterally to the stimulated nerve within the ventral lateral and medial subdivisions of the PBN, near the "waist" region, in experimental animals only. IXth nerve-activated neurons were located more medially and dorsally than VIIth nerve-activated neurons. In control as well as experimental animals, non-stimulus-related Fos labeling was present bilaterally in extreme, external, central, dorsal and superior lateral subdivisions, and in the external medial subdivision. This "background" labeling was increased over control levels only in the ipsilateral external medial subdivision in stimulated animals.

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Taste Specificity of c-Fos Induction in the Rat Nucleus of the Solitary Tract Following Conditioned Taste Aversion Formation. T.A. HOUPT, J.M. PHILOPENA, J.JHANG, T.H. JOH, and G.P. SMITH. (E.W. Bourne Behav. Res. Lab., Dept. Psychiatry, and Lab. Molecular Neurobiol., Dept. Neurol. Neurosci., Cornell Univ. Med. Coll.)

We have previously reported that c-Fos-like immunoreactivity (c-FLI) is induced in the medial intermediate nucleus of the solitary tract (NTS) in the rat by intraoral infusion of 5% sucrose previously paired 3 times with lithium chloride (LiCl) injections. Quinine sulfate may also be used as the conditioned taste stimulus: while quinine infusions are rejected and accompanied by aversive behavioral responses both before and after 3 pairings with LiCl, intraoral quinine infusions induce C-FLI in the medial intermediate NTS only after the pairings with LiCl in a distribution similar to that induced by sucrose following CTA formation. The current study was designed to determine if c-FLI in the NTS is specifically induced by the taste stimulus employed during CTA formation or if it is also inducible by an intraorally infused taste stimulus different from the conditioned taste stimulus. Rats were conditioned with either 5% sucrose or 0.3 mM quinine sulfate by 3 pairings of the intraorally-infused tastant (6 ml / 6 min) with systemic LiCl injection (150 mM, 12 ml/kg i.p.) 30 minutes later on 3 alternate days. One hour before sacrifice, rats conditioned with 5% sucrose received an intraoral infusion of quinine, and rats conditioned with quinine received an infusion of sucrose. The rats were transcardially perfused, and the NTS processed for c-FLI. Intake of sucrose dropped during conditioning trials in rats conditioned against sucrose, with no intake on the third pairing, and no quinine was consumed on the final test day. Rats conditioned against quinine rejected quinine during all trials, but did consume up to 6 ml of sucrose on the final test day. Only background levels of c-FLI was induced in the NTS of rats in either group. Thus quinine did not induce c-FLI in the NTS of sucrose-conditioned rats, and sucrose did not induce c-FLI in the NTS of quinine-conditioned rats. We conclude that both sucrose and quinine may serve as conditioned taste stimuli leading to c-FLI induction in the intermediate NTS, and that the c-FLI in the NTS is specifically induced only by the taste stimulus used during CTA formation.

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The Time Course of Taste Mixture Responses in Hamster Parabrachial Neurons. MARK B. VOGT and DAVID V. SMITH (University of Cincinnati College of Medicine).

Recently we have investigated the responses of third-order neurons in the hamster parabrachial nuclei (PbN) to binary mixtures of heterogeneous taste stimuli including sucrose, NaCl, citric acid and QHCl at different concentrations. While most mixtures evoked response frequencies that did not differ from those evoked by the more effective mixture component, the sucrose + QHCl and sucrose + citric acid mixtures evoked robust mixture suppression; i.e., the response to the mixture was significantly less than the response to the more effective component presented alone. We are now investigating the time course of these mixture responses in order to determine when mixture suppression occurs and how it relates to the time course of the responses to the individual components. Preliminary analyses indicate that response suppression first emerges during the 2nd post-stimulus second, reaches maximum magnitude by 3-4 seconds, and declines gradually thereafter until the stimulus is rinsed from the tongue. Interestingly, the period of maximum mixture suppression occurs *after* the period of peak response to the mixture or its individual components. This suggests a dissociation in the respective mechanisms that mediate the excitatory and suppressive effects of taste stimuli. In addition, we have observed that the temporal patterns of mixture responses differ in 'sucrose-best' and 'NaCl-best' neurons. These studies of response time course may provide insight into the neural processes mediating taste mixture responses in mammalian CNS neurons.

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Information processing in the gustatory nuclei of the brain stem: Electrophysiological data. PATRICIA M. DI LORENZO AND SCOTT MONROE (SUNY at Binghamton).

In previous attempts to characterize the processing of the neural signal related to gustation in the brain stem, comparisons of taste responses recorded at the nucleus of the solitary tract (NTS) and the parabrachial nucleus of the pons (PbN) have been made. Because these recordings were made at different times in the sample of units from each structure, it was not possible to draw conclusions about the time course of information transfer. In the present experiment, electrophysiological responses were recorded from pairs of cells, one from the NTS and a second from the PbN, in urethane-anesthetized rats. Taste stimuli included sapid solutions of NaCl (.1 M), HCl (.01 M), quinine HCl (.01 M), sucrose (.5 M) and Na-saccharin (.004 M). Averaged temporal patterns of evoked responses at both neural levels were analyzed as an approximation of the population vector associated with each stimulus. Results suggest that the temporal pattern of response of neurons in the PbN follows that of neurons in the NTS within different time frames for different taste stimuli. Changes in the across neuron patterns of response over time in both the NTS and the PbN were analyzed as vectors in an n-dimensional space, where n is the number of units tested. The response magnitude produced by each cell to a given stimulus provided the coordinates of the vector associated with that stimulus. The angle between two vectors provided an index of the similarity of the evoked across neuron patterns. By varying the interval over which the response was measured, it was possible to track the time course of angular separation of the across unit patterns as the response unfolded. In the NTS, the angles between across neuron patterns generally decreased over a 3 sec response interval, but in the PbN, these angles increased over time. If it is assumed that differences in the across neuron patterns associated with taste stimuli imply that information is being conveyed, then these data imply that neural activity in the NTS contains the most information in the earliest portions of the response while the information conveyed in the PbN intensifies during the later period of the response.

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Information processing in the gustatory nuclei of the brain stem: Gustatory Unit Stimulus Selective Topographical Organizer (GUSSTO), a network model. FRANK W. GRASSO AND PATRICIA M. DI LORENZO (SUNY at Binghamton).

Based on data derived from electrophysiological responses to taste recorded simultaneously in the nucleus of the solitary tract (NTS) and the parabrachial nucleus of the pons (PbN), three hypotheses about information processing in the brain stem were proposed: 1. across neural patterns in the NTS are most different in the earliest parts of the taste-evoked response, 2. the NTS transfers only the information in this initial portion of the response to the PbN, and 3. following this early transfer, the PbN elaborates the information about different tastes independent of NTS activity. A neural network model, called the Gustatory Unit Stimulus Selective Topographical Organizer (GUSSTO), was constructed which was designed to emulate these features. GUSSTO implements two levels of processing, analogous to the NTS and the PbN. The input from a "receptor" sheet was mapped with modifiable excitatory connections to processing units at the "NTS" level. Within the "NTS", processing was influenced by fixed lateral inhibitory connections. Units at the level of the "PbN" received fixed excitatory connections from the "NTS" and make plastic inhibitory connections within the layer. GUSSTO was trained with simulations of responses analogous to those produced by the four "basic" taste qualities, i.e. sweet, sour, salty and bitter, by the chorda tympani nerve. GUSSTO was permitted to self-organize a representation of "taste stimuli" based on this experience. A Hebbian learning rule was used to modify connections. Preliminary results have demonstrated that, over the course of training, both the "NTS" and "PbN" layers acquire relative specializations with respect to the array of "tastants" tested, but retain the ability to respond broadly across stimuli. Over several time steps following the initial influx from the receptors, most of the "NTS" activity becomes concentrated in a few very specialized cells. However, at the level of the "PbN", once the initial input from the "NTS" is received, the responses continue to elaborate without further guidance from the "NTS".

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A Computerized Gustatory Stimulator That Delivers Taste Solutions Through a Single Nozzle Without Contamination EVEY L.A., and NORGREN R. (Pennsylvania State University, Hershey, PA 17033)

Experiments with taste require delivery of a fluid stimulus to the receptor surface. Presenting fluids through separate nozzles increases variability. A single nozzle reduces this variability. Requirements of this nozzle are a precise aim, a constant flow rate, and no contamination. We developed a computerized gustatory stimulator that meets these requirements. A key feature is a vacuum drain that diverts fluid away from the receptor surface. This drain makes it possible to flush the system between trials. There is a concentric vacuum chamber surrounding the stimulus nozzle. At one end there is an opening that receives a vacuum tube. At the other end there is a small funnel shaped opening for the tip of a fluid nozzle. Fluid reaches the receptor surface when the tip is outside the chamber. Fluid reaches the vacuum drain when the tip is inside the chamber. A miniature pneumatic cylinder extends and retracts the nozzle through a linear movement of about 1 cm. The extended nozzle fits tightly into the funnel shaped opening. This shape forms a seal and precisely aims the nozzle toward the stimulation site. The seal prevents vapors within the chamber from reaching the receptor surface. The tip of a water rinse nozzle extends out of the chamber when the stimulus nozzle is inside. A 386DX or faster computer is necessary for the software to run in *Microsoft Windows 3.1*. The computer language chosen for the stimulator software was *Microsoft C70*. Operation of the stimulator is under menu control. The stimulator program runs as a "windowed" DOS application in *Microsoft Windows 3.1*. Our data acquisition program runs simultaneously, also as a "windowed" DOS application. Fluid delivery and data acquisition are under the control of one computer. The gustatory stimulator has an added advantage for neural recording because automation makes it unnecessary to disturb the preparation, thus, enhancing stability, eliminating vibration, and reducing electrical noise.

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Reflex Connections between a Primary Gustatory Nucleus and Motoneurons controlling oromotor activity.

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Previous studies indicate that in goldfish, neurons with glutamate-immunoreactive axons form a connection between the primary gustatory sensory zone of the vagal lobe and the underlying motoneurons that innervate the oropharynx. The current studies were undertaken in order to characterize the neurons involved in this reflex connection and to determine which of these have high-affinity glutamate uptake mechanisms characteristic of glutamatergic neurons. HRP or biotinylated dextran amines were used to retrogradely label those neurons projecting to the layer containing the oropharyngeal motoneurons. Similarly, tritiated D-aspartate was used to label radioactively those neurons with a high affinity glutamate uptake system. For these experiments, 500-600 μm slices of the vagal lobe were prepared on a vibratome and permitted to stabilize in physiological Ringer's solution. The tracers were injected into the vicinity of the motoneurons and the slices were permitted to stand for an additional 4-6 hours. After this time, the slices were fixed in 0.05% paraformaldehyde and 3% glutaraldehyde in phosphate buffer. The slices were cryoprotected and re-sectioned on a freezing microtome at 100 μm thickness. After reacting with standard ABC-peroxidase and DAB methods, the tissue was embedded in plastic and sectioned at 5 μm for autoradiography. Most cells projecting to the motor zone were situated in sensory layers 5 and 7 although a few neurons in layer 3 also were labeled. Autoradiography reveals that glutamatergic neurons lie in all of these layers. Results of double-label studies, now underway, will show whether all of the reflex system neurons are also labeled by the tritiated D-aspartate.

Forebrain Projections from the Gustatory Cortex of the Syrian Golden Hamster.

R.G.WEHBY, J.deB. ZEIGER, and J.A.LONDON (Center for Neurological Sciences and Department of BioStructure and Function, Univ. of Connecticut Health Center, Farmington, CT 06030)

We describe here some results from our ongoing investigations on the anatomy of the gustatory cortex of the Syrian golden hamster (*Mesocricetus auratus*). Biotinylated dextran (10kD, 5%, 2-10 µl, 6 animals) was pressure-injected into either the gustatory cortex, or into regions dorsally- or ventrally-adjacent to the gustatory cortex. We define the gustatory cortex as that region of insular cortex in which taste responses from anterior or posterior tongue stimulation have been elicited in previous experiments (Wehby and London, 1993; London, 1990). At the injection site the biotinylated-dextran-filled cells resembled Golgi-impregnated cells. Anterogradely-labelled axonal and dendritic arborizations, as well as dendritic spines, were clearly visible, even in sections counterstained with thionin. An injection into the rostral region of the gustatory cortex (approximately 500µ rostral to the genu of the corpus callosum) demonstrated projections from this region to the ipsilateral infralimbic cortex, amygdala, lateral hypothalamus, and contralateral gustatory cortex. An injection at a more caudal site in the gustatory cortex (at the level of the genu of the corpus callosum) had a similar projection pattern, but with a relatively sparser projection to the ipsilateral infralimbic cortex. In contrast, two injections located dorsal to the gustatory cortex (in somatosensory cortex), one at the level of the genu of the corpus callosum and one 500µ rostral to the genu of the corpus callosum, revealed projections to ipsilateral and contralateral somatosensory areas. Two injections located ventral to the gustatory cortex (in dorsal piriform cortex, at the level of the genu of the corpus callosum) revealed heavy projections to the ipsilateral amygdala and piriform cortex, as well as sparser projections to the contralateral piriform cortex. These studies demonstrate that the projections from the gustatory cortex of the hamster communicate with limbic and autonomic areas of the forebrain, and that this projection pattern is different from that of adjacent cortical areas.

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Central Connectivity of the Hamster Gustatory Nerves Demonstrated with Biotinylated Dextrans.

A.P. KNOX, M.A. BARRY (UCONN Health Center, Farmington, CT)

We were interested in taking advantage of the superior labeling properties of biotinylated dextrans to demonstrate the terminal fields of hamster gustatory nerves as an adjunct to immunocytochemical studies. We studied the central connectivity of the cranial nerves that carry taste information including the chorda tympani (CT) branch of the facial nerve of adult-male golden Syrian hamsters using biotinylated dextrans (BD) (3 kDa and 10 Kda, Molecular Probes). After exposure of the CT in the middle ear, BD crystals were applied to the cut central end of the nerve. Survival times varied from 2-21 days. The ABC reaction (Vector) was used to reveal transported BD in either transverse or horizontal 50 μm brainstem sections. Several fascicles of transganglionic (anterograde) labeled fibers entered the rostral medulla and extended caudally to the level of the area postrema/commissural nucleus. Retrogradely labeled cells were confined to the preganglionic parasympathetic cells of the superior salivatory nucleus (SS). Terminal label was extremely dense at the rostral pole of the solitary nucleus (NTS) and very dense at more caudal levels of the rostral NTS where it overlapped with the medial and presumably gustatory terminal field of the glossopharyngeal nerve. The 3 Kda BD appeared qualitatively superior to the 10 Kda BD with regard to the extent of dendritic filling in SS and terminal label throughout the brainstem. For anterograde labeling, BD was superior to other tracers such as Cholera toxin, HRP, and WGA-HRP because of the rapid transport, high sensitivity, temporal stability, and particularly the "Golgi-like" character of the label.

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Development of the Vagal Gustatory System in the Goldfish.

CHARLES F. LAMB (Dept. Cell. & Struct. Biol., Univ. Colorado Hlth. Sci. Ctr., Denver CO).

The goldfish vagal lobe is a laminated enlargement of the dorsal medulla consisting of superficial sensory layers overlying a layer of motoneurons. To understand the ontogenetic development of this complex structure, I studied the temporal relationship between the development of pharyngeal taste buds, the vagal ganglia and nerve, and the vagal lobe. Early embryos (<24 hrs old) were acquired commercially and raised to 35 days in the laboratory (at 21° C). Specimens were collected every 6 hrs until hatching, then every 12 hrs, and were fixed in 4% paraformaldehyde for subsequent analyses (silver-staining, immunohistochemistry, or DiI labeling). DiI was applied to the palatal organ or vagal ganglion, after which the fish was sectioned to identify both central and peripheral vagal fibers. Goldfish hatched at approximately 4.5 days (all times post-fertilization), and were actively swimming and feeding by 6 days. At hatching, although the visual, olfactory, and acoustico-lateralis systems are relatively well-developed, the gustatory system is immature. The vagal ganglion forms at 3 days, with fibers extending to the medulla and pharynx by 4 days. The fibers enter the pharyngeal epithelium and are associated with immature taste buds by 5 days. By 1 week, the palatal organ is increasing in thickness and taste buds are maturing. DiI applied to the vagal ganglion at this stage transcellularly labels cells in pharyngeal taste buds. At this time, the vagal lobe is evident only as a region of heightened cell proliferation on the dorsolateral surface of the medulla. By 3 weeks, the vagal lobe protrudes from the medullary surface and motoneurons can be labeled by DiI applied to the palatal organ; also, mature taste buds are present in the pharynx. By 4 weeks, the sensory region of the vagal lobe begins forming, and the gustatory epithelium is thickening. Although separate sensory, fiber, and motor regions of the vagal lobe are apparent by 5 weeks, no obvious lamination is present. Cell proliferation occurs at the dorsal tip of the vagal lobe as the lobe grows dorsomedially over the surface of the medulla.

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Development of Intrinsic Neurophysiological Properties of Cells in Rat Nucleus of Solitary Tract Using an In Vitro Slice Preparation. H. BAO, R.M. BRADLEY and C.M. MISTRETTA (School of Dentistry, University of Michigan, Ann Arbor, MI 48109).

Developmental alterations in taste responses and morphology of gustatory cells in the nucleus of the solitary tract (NST) are well documented in rat. However, there is no information about the maturation of intrinsic electrophysiological properties or repetitive discharge patterns of neurons in the gustatory zone of NST to indicate when the cells are first functional. Therefore we used whole cell recordings in an in vitro slice preparation of the rostral brainstem to learn when the intrinsic electrophysiological properties of second order neurons mature, and to study relations among development of intrinsic properties, neurophysiological taste responses and morphological characteristics. NST cells from rats in six age groups were studied: 5 days postnatal (13 cells); 10 days (14 cells); 15 days (19 cells); 20 days (18 cells); 30 days (10 cells); and, adult (11 cells). Resting membrane potentials did not differ during development and averaged from -55 to -60 mV across groups. However, input resistance decreased from about 800 MΩ at 5 days postnatal to 435 MΩ in adult, and membrane time constant, determined with a single exponential, decreased from 48 to 20 msec. In addition, action potentials from immature neurons were both slower in rate of rise and longer in duration than those from mature cells. In both immature and mature cells, injection of larger current pulses elicited a higher discharge frequency, but slopes for discharge frequency versus current strength tended to be steeper in older neurons. At all ages there was a clear accommodation in frequency of the spike discharge. Furthermore, neurons could be identified at each age with intrinsic spike discharge patterns characteristic of Groups I, II, III and IV that were previously described in adult rat NST. The developmental differences in input resistance and membrane time constant, and in action potential rate of rise, duration, and frequency all plateau in cells from the 15 or 20 day postnatal age groups. Therefore, maturation of several intrinsic neurophysiological properties of cells in NST is essentially complete by about 3 weeks after birth. This is well in advance of the more prolonged postnatal development of extracellular responses to taste stimuli and some aspects of dendritic morphology.

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Alterations in the Distribution of Geniculate Ganglia Proteins Following Early Postnatal Receptor Damage. PHILLIP S. LASITER* and BERNARD B. BULCOURF (*Florida Atlantic University*)

Orochemical stimulation of fungiform receptor systems during the first week of rat's postnatal life induces normal development of primary gustatory axons in the rostral nucleus of the solitary tract (NST). To examine the biochemical correlates of normal axonal development within the NST, we have conducted studies that examine potential changes in the distribution of proteins within geniculate ganglia following early postnatal receptor damage. Rat pups received unilateral receptor damage to fungiform papillae at postnatal day 2 (P2), similar to that described in our previous experimental anatomical studies. Following damage and recovery, geniculate ganglia ipsilateral and contralateral to damage were removed, proteins were solubilized, and samples were used in two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Gels were silver-stained, and quantitative densitometric analyses were conducted. Western blots were also conducted to verify the identify of various proteins. Ages of samples ranged from P7 to P38, which allowed us to analyze both developmentally-related changes in the distribution of proteins, in addition to alterations in protein distribution that were specifically caused by receptor damage. Staining density of four proteins within the 18-30 kD range (designated as C1, C2, C5 and C8: *pI* range; 4.4 - 5.6) was significantly lowered by receptor damage, one of which (C2) was identified as calbindin-D. Staining density of seven proteins within the 30-60 kD range (designated as B1, B3, B7, B8, B14, B17 and B18: *pI* range; 4.3 - 6.2) was also initially lowered by receptor damage, one of which (B8) was identified as GAP-43. Two proteins within the 60-250 kD range (designated as A1 and NF200: *pI* range; 4.3 - 4.6) were affected by receptor damage. Staining density of all proteins except GAP-43 and A1 returned to normal (contralateral) values by P38. Therefore, the distribution of two growth-associated proteins (GAP-43 and protein A1) were irreversibly lowered as a consequence of early postnatal receptor damage. Protein A1 has not apparently been characterized in previous studies, and it has a differential distribution in tissues that we have examined. A1 is not present in optic nerve nor cervical spinal cord, but it is present in geniculate ganglia, facial-intermediate nerve, fungiform papillae, gasserian ganglia, and cerebellum. A1 is not present in liver. Thus, A1 does not appear to be a glial protein, nor a general 'metabolic' protein. We are currently conducting immunohistochemical studies to localize protein A1 in central and peripheral tissue.

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The Effect of Food Deprivation on Gustatory-Evoked Activity in the Nucleus Tractus Solitarius of the Rat

LAURENCE. J. NOLAN and THOMAS R. SCOTT
(University of Delaware)

The results of human psychophysical and animal behavioral studies suggest that a state of food deprivation alters hedonic perception of taste stimuli and broadens the range of substances an animal will accept as food. We recorded from the NTS of 72-hour deprived rats to determine whether these hedonic and behavioral changes resulted from alterations in the sensitivity of the taste system. The deprivation resulted in a 10% decrease in body weight at the time of recording. The evoked activity of 45 units from food deprived rats and 40 units from calorically replete rats to an array of 13 taste stimuli was recorded and analyzed. Food deprivation significantly reduced the spontaneous firing rate of NTS gustatory neurons and attenuates the response to the sodium and lithium salts. In addition, the breadth of tuning of salt-sensitive neurons was greater in food deprived rats than in controls. We then analyzed for differences in the code for taste quality. Activity profiles evoked by the salts (NaCl, LiCl, MSG) and carbohydrates (Polycose, glucose, fructose, sucrose) were significantly more similar in deprived rats, resulting in a contracting of the space. The implication is that deprived rats are able to make fewer discriminations among tastants, an effect that could underlie their wider acceptance of potential foods.

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Picture-based Odor Identification in Alzheimer's Patients and Normal Controls. CHARLIE D. MORGAN (San Diego State University), CLAIRE MURPHY (San Diego State University and UCSD Medical Center), STEVEN NORDIN (UCSD Medical Center and San Diego State University)

The purpose of this study was to determine the difference in ability to identify odors between Probable Alzheimer's patients and normal controls using the picture-based San Diego Child-Odor-Identification Test. Conventional odor identification tests, such as the UPSIT, consisting of written words as response alternatives, have previously demonstrated poor odor identification performance in patients with Alzheimer's disease (AD). By using the children's test the cognitive load produced by these words is lessened. All subjects were participants in the UCSD Alzheimer's Disease Research Center (ADRC) on-going study of olfactory function and had been diagnosed either as having Probable AD or as normal by two independent neurologists at the ADRC using the NINCDS-ADRDA diagnostic criteria. AD patients had mild or moderate dementia as assessed by the Dementia Rating Scale. The stimuli used were peanut butter, chocolate, coffee, playdough, baby powder, bubble gum, cinnamon, and mustard presented in opaque jars. A set of 20 visual stimuli (the eight odor items and twelve distractors) in an array was placed in front of the subject from which s/he was to choose. To ensure that the visual stimuli were familiar, the subject first identified these pictures before being presented the odors. The subject was instructed to close a nostril with a finger as well as both eyes before sniffing from the jar. All eight odors were presented to each nostril in a random order, alternating nostrils. The inter-stimulus-interval was 45 seconds to avoid adaptation. If unsure, the subject was required to guess. Results showed that Probable Alzheimer's patients were significantly poorer at identifying odors than normal controls ($p<.05$). These findings suggest that the poor odor identification performance in AD reflects a true decline in identification ability, rather than semantic difficulties in interpreting written words.

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Deterioration of Implicit Long-Term Olfactory and Visual Memory in Alzheimer's Disease. CAPRICE A. NICCOLI, LETICIA ACOSTA (San Diego State University), STEVEN NORDIN and CLAIRE MURPHY (UCSD Medical Center and San Diego State University)

The key diagnostic factor of Alzheimer's disease (AD) is the identification of neuritic plaques and neurofibrillary tangles, which have been found to a large extent in the olfactory pathways and in the cortical areas involved in memory functioning. Based on these finings, it may be expected that long-term memory for odors may be more affected than that for other sensory modalities. One approach to assess long-term memory is through rated familiarity with a stimulus, as it can be assumed that familiarity reflects recognition memory for the occurrence of a stimulus in a persons past. In the present work, implicit long-term memory, operationally defined as rated familiarity, was studied in patients with probable AD and in healthy elderly controls who were diagnosed by two independent neurologists at the UCSD Alzheimer's Disease Research Center. The subjects were presented with olfactory and visual stimuli and were asked to rate each stimulus on the basis of its familiarity on a 160 mm bipolar visual-analog scale ranging from "Not Familiar" to "Very Familiar". The stimuli used were 15 common household odors, 50 faces of US presidents and vice presidents, 50 abstract engineering symbols, and 10 colors. A randomly selected sample of 10 stimuli of each stimulus type was presented to the subject. The results show that the AD patients rated all types of stimuli as significantly less familiar than did the normal controls. Importantly, the most disparate ratings occurred with the olfactory stimuli. These results imply that long-term memory for olfactory and visual stimuli is affected by the disease, with the greatest deterioration occuring in memory for odorants. These results are important for the understanding of the disease process and for its diagnosis.

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Olfactory Dysfunction: Clinical Issues in Brain Injury Rehabilitation. RICHARD M. COSTANZO (Virginia Commonwealth University) and NATHAN D. ZASLER (National NeuroRehabilitation Consortium).

Eighty-seven individuals with traumatic brain injury were evaluated at the MCV Smell and Taste Center over a 4 year period. Severity of neurologic injury was assessed with the Glasgow Coma Scale (GCS), indicating 49% severe (GCS 3-8), 31% moderate (GCS 8-12), and 20% mild (GCS 13-15). Olfactory function testing demonstrated 31% of the population to be anosmic, 54% dysosmic and 15% normosmic. There was a correlation between severity of injury and olfactory function scores. Interestingly, 10 percent of the patients experienced parosmias. Investigators will discuss their experience in evaluating and treating this unique population including: (1)neurodiagnostic strategies such as static and dynamic functional imaging; (2) assessment of impairment and disability within the clinical and legal context; (3) rehabilitative treatment issues including compensatory strategies to insure safety, improve quality of life and optimize vocational and avocational reentry. Case presentations dealing with olfactory phantoms and distortions will be presented. Given the high incidence of head trauma in the United States (over 2 million cases per year), it is important for clinicians to be aware of the potential impairment and disability associated with post-traumatic olfactory dysfunction.

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Chemosensory Assessment of HIV-Infected Adults. RICHARD D. MATTES, Charles J. Wysocki (Monell Chemical Senses Center), Amy Graziani, Rob Roy MacGregor (Hospital of the University of Pennsylvania).

Alterations of taste and smell have been reported in HIV-infected patients. Sensory changes could stem from nutrient deficiences, oral complications of HIV infection, including oral candidias and burning mouth syndrome as well as therapeutic pharmacologic agents. The aims of this preliminary study were to evaluate the nature, severity and nutritional implications of chemosensory abnormalities in patients with HIV infection. In a single test session, 25 male patients were administered the odor identification test and rapid screening taste test used in the Monell-Jefferson Taste and Smell Center and performance was compared to the normative data base established at the Center. Patients also were tested for sensitivity to androstenone. Following psychophysical testing, body weight and composition were determined and dietary information was obtained by questionnaire. On a separate day, patients were given physical exams including biochemical evaluations. Patients ascribed significantly higher intensity ratings for the sucrose, citric acid and quinine samples relative to the controls. Twelve percent of patients had odor identification scores below the normal range forthe clinic. Chemosensory function was not significantly associated with treatment drug, age, weight, weight change, body composition, CD4+ cell count, hemoglobin, hematocrit, liver enzyme levels, duration of infection, or other infection-related complications (e.g., diarrhea, night sweats, fevers). Although the sample size is limited, the data fail to support a role of significant chemosensory changes in the poor appetite often associated with HIV-infection or an adverse effect of infection on the chemical senses.

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Olfactory Deficits in Patients Infected with the Human Immunodeficiency Virus. DAVID E. HORNUNG, DONALD C. BLAIR, ELIZABETH C. CLARK, PAUL R. SHEEHE, and DANIEL B. KURTZ (SUNY, Health Science Center, Syracuse, N.Y. 13210)

The 40 item University of Pennsylvania Identification Test (UPSIT) was administered to 110 HIV positive patients seen in the SUNY Immune Deficiency Clinic. Fifty seven percent of those tested showed a reduced olfactory ability (55th percentile or less) relative to age and sex matched normative data. The observed olfactory losses were examined with respect to various epidemiological and clinical factors associated with AIDS. The presence of AIDS dementia, sex, and age were all found to be associated with the olfactory loss. The olfactory losses were more severe in patients with AIDS dementia as compared to HIV-positive patients who did not show any symptoms of CNS involvement. Older subjects were more likely to exhibit the loss than were younger subjects, and women were more likely to have a reduced ability as compared to men. Heterosexual patients who were infected by their partners and IV drug users were more likely to show a reduced olfactory ability as compared to homosexual men or patients who were infected by transfusions or needle sticks. Preliminary longitudinal data suggest that impaired olfaction might serve as a marker and perhaps even a predictor of a developing AIDS dementia.

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Discrepancies in Perceived and Diagnosed Smell Sensitivity in Patients with Alzheimer's and Nasal/Sinus-Inflammatory Disease, and in Normal Elderly. DAYNA WILHITE (San Diego State University), STEVEN NORDIN and CLAIRE MURPHY (San Diego State University and UCSD Medical Center)

The purpose of this study was to determine how perceived smell sensitivity is related to diagnosed sensitivity, and whether this relationship differs among 80 patients with probable Alzheimer's disease (AD), 77 patients with nasal/sinus-inflammatory disease (NSID), and 79 normal elderly. Perceived smell sensitivity was assessed by questionnaire with the response categories: "Normal", "Decreased", "Complete Loss", and "Increased". Smell thresholds were determined for butanol by the ascending method of limits with 10 dilution steps. Each successive step was one third the concentration of the preceding. In this specific study, we defined a smell loss to be present at Dilution Step 5 (19 ppm) or below. The results showed that the AD (Dilution Step 3.8) and NSID (3.9) patients both had significantly higher thresholds (lower dilution steps) than the normal elderly (6.0). For those subjects who all reported a "Normal" smell sensitivity, the average threshold in dilution steps was 4.1 for the AD patients, 7.1 for the NSID patients, and 6.4 for the normal elderly, which differed significantly among the AD patients and the other two groups. Focusing on the subjects with a diagnosed smell loss, "hit" rates ("Decreased" or "Complete Loss"/diagnosed loss) and "false-alarm" rates ("Decreased" or "Complete Loss"/diagnosed as normal) were determined for each group, which were 28% and 13% for the AD patients, 98% and 81% for the NSID patients, and 24% and 9% for the normal elderly, respectively. Chi-square analyses showed that both the "hit" and "false-alarm" rates for the NSID patients were significantly higher than those for the other two groups, the two latter groups not differing significantly. However, it should be noted that the AD patients and the normal elderly have about the same tendency to report a smell loss despite the fact that the AD patients have markedly higher thresholds. In conclusion, these findings suggest that the AD patients are, in general, not aware of their pronounced loss in smell sensitivity, which to some extent also holds for the normal elderly. The NSID patients, on the contrary, have a very strong tendency to report a smell loss.

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Changes in Olfactory Ability During the Course of an Upper Respiratory Infection KAREN CHOJNACKI, DAVID E. HORNUNG, DANIEL B. KURTZ, AND KARL MC KNIGHT (Biology Department, St. Lawrence University, Canton, N.Y. 13617 and SUNY Health Science Center, Syracuse, N.Y. 13210)

To determine the impact of an upper respiratory infection (URI) on olfactory ability, 15 college students who had been identified as having URIs were administered the Odorant Confusion Matrix at various times throughout the course of their colds. Thirteen of the 15 subjects showed at least a 20 point increase in their OCM scores during the course of recoveriy from the URI suggesting that olfactory ability was indeed impaired as a consequence of the infection. However, the reduced olfactory ability was not equal across all the odorants of the OCM. The odorants with low water solubility (orange and mothballs) were relatively easily identified, even on the days with the poorest overall olfactory performance (lowest overall percent correct). In contrast, odorants with high water solubility (ammonia and rubbing alcohol) were poorly identified during the height of the infection. It is hypothesized that the mucosal swelling, which usually accompanies a cold, accentuates the difference between highly and poorly sorbed odorants in the loss of odorant molecules to non-olfactory tissue. This data also suggest the possibility that certain disease processes can produce distinct alterations in the patterns of errors in the OCM which may be helpful in the diagnosis of olfactory dysfunction.

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Nasal Disease and Olfaction in Chronic Sinusitis Patients. MARITESS MAURICIO, TERENCE M. DAVIDSON, ALFREDO A. JALOWAYSKI (UCSD Medical Center) and CLAIRE MURPHY (UCSD Medical Center and San Diego State University)

Sinusitis is the most common chronic illness in the United States, affecting one in every eight persons. Although symptoms of chronic sinusitis (e.g., nasal congestion and obstruction, post-nasal drip, and facial pain) are well documented, the impact and manifestations of the disease on olfaction has not been thoroughly investigated. This study attempts to further define the state of olfactory function and nasal disease as it exists in patients with chronic sinusitis before and after endoscopic sinus surgery (ESS) at the UCSD Nasal Dysfunction Clinic. All subjects gave a careful history of their illness, including their symptoms, and were allowed to self-evaluate their present conditions using a scale of 0-100. Standard blood work for IgE as well as CT scans were obtained prior to ESS. Odor threshold and performance on odor identification was assessed as well as nasal physiology, cytology and endoscopy. Results indicate a significant improvement in olfaction after ESS ($p<.01$). Chronic sinusitis symptoms of nasal congestion and obstruction, post-nasal drip, and facial pain showed marked improvement, as reported by the patients three months post-surgically ($p<.001$ in all cases). Furthermore, allergy-related symptoms, which may promote the development of chronic sinusitis, were also found to correlate with olfactory performance: obstruction of the ostiomeatal complex ($p<.0001$), IgE scores ($p<.001$), and cytology scores (e.g., basophils, neutrophils, and goblets; $p<.0001$). In conclusion, these results show that ESS can be successful in improving olfactory functioning in patients with chronic sinusitis.

Supported by NIH grant AG04085 (CM).

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Olfactory Loss in a Brother and Sister Following Their Simultaneously Contracted Upper Respiratory Viral Infections: A Case Report.
ALAN R. HIRSCH (Smell & Taste Treatment and Research Foundation)
DANA OSTER (University of Illinois Medical School, Chicago)

Olfactory loss most commonly originates with an upper respiratory viral infection. We report the case of a brother and sister both of whom developed chemosensory losses following upper respiratory viral infections which they contracted simultaneously while sharing the same household with other family members who developed neither respiratory infections nor chemosensory losses. The brother, a 53-year-old business manager, and the sister, a 50-year-old photographer, both white and right handed, occupied the same house for 2 weeks in April, 1991. Both siblings reported having had coryza, rhinorrhea, weakness, sternutation, low-grade fever and fatigue at that time. During the following 1 to 2 weeks, both noted an inability to smell and taste. Despite persistence of these complaints, both siblings now experience gustatory and olfactory windows. The existence of an olfactotropic viral infection is supported by (1) the olfactory point source of chemosensory dysfunction, (2) the temporal course of the siblings, (3) the similar presentations of brother and sister and (4) the occurrence of olfactory windows which have been reported after viral infections. We suspect that olfactory loss following viral infection is an underreported phenomena since (1) physicians do not routinely inquire about chemosensory loss or test for it, (2) patients themselves are unlikely to recognize the problem or seriously address it, and (3) patients are unlikely to discuss this problem casually.

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Olfactory Threshold in Allergic Rhinitis Patients Before and After Nasal Allergen Challenge ALFREDO A. JALOWAYSKI (UCSD Medical Center), KRISTEN KONAR (San Diego State University), TERENCE M. DAVIDSON (UCSD Medical Center), CLAIRE MURPHY (UCSD Medical Center and San Diego State University)

Patients with allergic rhinitis often complain of impaired sense of smell. In this study we evaluated the effects of early-phase allergic reaction, characterized by nasal itching, sneezing and obstruction, on olfaction and nasal air flow. N-Butanol olfactory threshold was measured in a small group of allergic rhinitis patients before and after nasal allergen challenge as an objective measurement of olfactory function. In this study, allergic rhinitis patients were nasally challenged with increasing concentrations of allergens until their nasal airflows decreased by 50% or more from baseline values. An anterior rhinomanometric method was used to evaluate nasal patency, in addition to counting the number of sneezes after each challenge. A calibrated nebulizer was used to deliver 0.4 ml of cat, mite or grass mixture antigens. Thresholds were measured using a forced-choice, two-alternative ascending method, in which incorrect choices led to the presentation of a higher concentration and correct choices led to the presentation of the same concentration, until the subject reached a criterion of 5 correct successive choices. As expected, the results indicate that all allergen challenged patients had a significant decrease in nasal airflow, $p < .05$. However, an Analysis of Covariance, using rhinomanometry as the covariate, suggests a trend towards olfactory thresholds increasing after exposure to an antigen. One possible explanation may be that a decreased nasal patency may enhance olfaction due to increased turbulent airflow. Histamine, known to be released intranasally after allergen challenges and to cause allergic symptoms, may also affect or modulate olfaction.

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The Effect of Floral Odor on Learning.

ALAN R. HIRSCH (Smell & Taste Treatment and Research Foundation)
LISA H. JOHNSTON (Rush-Presbyterian-St. Luke's Medical Center)

Various studies have evaluated the effects of odor on behavior, but none has systematically assessed the effect of odor specifically on learning. In order to do so, we evaluated the learning ability of twenty-two volunteers, twelve males and ten females ranging from 15 to 65 years of age (mean 36 median 34). All subjects took the Chicago Smell Test and the Pyridine Threshold Test of Amoore to establish that their olfactory ability was normal. Subjects then underwent testing in radmonized single blinded fashion, with two trail-making (maze) tests modified from the trail-making subtests of the Halsted-Reitan Neuropsychological Test Battery. All subjects underwent trial twice: once while wearing a floral-scented mask and again while wearing an unscented mask. Prior to testing, patients wore the masks for 1 minute to minimize their distracting effects. The order of presentation of scented versus unscented masks was random, but the order of maze presentation was constant and each subject attempted the set of two mazes a total of 3 times sequentially with each mask. The time required to complete each trial was measured. The percent change in the time required to complete the second and third trials compared to the first trial was analyzed using Man-Whitney-U, Spearman rank correlation, and Wilcoxon rank sum tests for nonparametric data. Subjects with normal olfactory ability who considered the odorant hedonically positive demonstrated that on subsequent trials they learned to complete the tasks 17% faster on average in the presence of the floral odor than in the nonodorized condition. These findings imply that future studies may validate the use of odors as adjuvants to learning and education as well as rehabilitation.

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Specific Hyperosmia: Fact or Artifact?

JOHN E. AMOORE (Olfacto-Labs).

Olfactory detection threshold measurements on human observers, using the "sniff test" method from odorant solutions in glass flasks or plastic squeeze-bottles, occasionally identifies individuals who perform extraordinarily well with a particular chemical. In terms of the decismel scale, they can distinguish the test odor from a blank at -25 dS or less (18 times more sensitive than the mean normal threshold in the population, defined as 0 dS). This is over 2 SD more sensitive than the norm, and is to be expected in not more than 2% of a normal distribution. Such observers are typically mediocre in their sensitivity to other odors. This phenomenon, if confirmed, might be termed "specific hyperosmia", and is of substantial practical and theoretical interest. We have encountered numerous potential examples, but many proved to be the result of experimental errors or method artifacts, or were simply unrepeatable. Some illustrations will be provided of probably false hyperosmias, and also of some candidates that are likely real, or at least not yet disproved. Two examples that we have under investigation will be described. The standard odorant n-butyl alcohol has a solvent or lacquer odor. Among 31 subjects doing squeeze-bottle threshold tests we found three that could reliably distinguish the -30 dS level. One of them commented that the odor was like cheese. We suspect that there has been a partial oxidation of n-butyl alcohol to n-butyric acid (which has a substantially lower detection threshold) in the nasal cavities of these extra-sensitive subjects. Another example is alpha-chloroacetophenone, a lachrymatory. We have encountered one (anosmic) subject who could detect it repeatedly at the -25 dS level by puffing the vapor toward her eyes. This suggests a trigeminal nerve hyperesthesia (the mean normal eye irritation threshold is at 120 times higher concentration).

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Influence of synthetic Acids on Mens Assessment of Women

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In two double blind studies the effects of a fatty acid mixture and p-hydroxyphenylpropionic acid on the assessment of women by men have been investigated. In study I 77 men rated 5 women on bipolar adjective scales.The mixture of acids consisted of 9.2 µg acetic acid, 8.8 µg propionic acid 4.2 µg iso-butyric, 12.8 µg n-butyric, 8.3 g iso-valeric acid dissolved in ethanol. In primates this mixture and p-hydroxyphenylpropionic acid (HPPA) function as attractants (copulin). In group I the mixture, in group II HPPA, in group III the solvent was applied on the upper lip of each subject. In group IV no odour was applied. In the beginning of the session an instruction was handed out. After this a standardized questionnaire concerning the subject state of mind was filled in. One substance was applied on the upper lip (except in the no odour group). The five pictures of women were rated one by one (random order) on 28 adjective scales. In comparison to the no odour group the women were assessed as significantly (alpha adjusted) more attractive, more sportive, more anticing anziehend, less expressive, the men like them more but do not want to meet them. Under the mixture the overall physical attractivenes of the women was estimated as higher. HPPA has only a significant effect on the rating of overall physical attractiveness. There was no significant difference between ethanol an the no odour condition.

In study II the mixture and phenylethyl alcohol (0.01 v/v %) (control odour) and aqua dest. (control) have been tested. 76 men took part in this study with the same procedure as in study I. In comparison to aqua dest. the women were rated under the influence of the mixture as more sensitive, more attractive more interesting but less expressive. Phenylethyl alcohol had no significant effect on the ratings in comparison to aqua dest.

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Odor Qualities of Androstenone and Pemenone as Perceived by Pemenone-Osmics and Allosmics. DAVID A. STEVENS (Clark University) ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology)

The validity of the odor quality reports given by human subjects is often questionable. On the one hand, social conventions can influence the labeling of putrid or uncommon qualities, and on the other, semantic differences exist for odor descriptors among individuals. Our current work centers on individual differences in the odor qualities of androstenone (AND) and pemenone (PEM). Here we sought to establish empirical support for the differences in odor quality reports, using a non-verbal, semantic-free method. Undergraduate volunteers sniffed a swab containing 150 μl of 390 μM PEM, rated its intensity on a 9-point scale, and reported the quality of its odor. They were classified as osmic (N=42) if the quality report was putrid (urine-like, sweaty), allosmic (N=23) if it was non-putrid, and anosmic (N=38) if no odor was detected. The subjects then rated individual swabs, each containing 150 μl of one of the 15 odorants at concentrations judged to be about equally strong by another panel of PEM osmics and allosmics. The odorants were the modally putrid AND, butyric, caproic and isovaleric acids, and PEM, the woody or vegetable-smelling basil, celery, pepper pyrazine, galbanum, and pinene, and the floral or fruity-smelling lavender, muget, phenylethyl alcohol, citralva, and orange. Both AND and PEM are usually characterized as putrid by osmics but some subjects describe PEM with vegetable or floral descriptors, and some report no odor. Subjects independently sorted all of the odorants into groups such that each member of a group had the same or a very similar odor quality. The subjects were allowed to form as many groups as they wished. The frequencies with which each of the different odorants was paired with the others were used as data for an independent multidimensional scaling by ALSCAL for each class of subject. Three-dimensional solutions showed that osmic subjects grouped AND and PEM with the other modally putrid odorants, whereas the allosmics grouped them with the vegetable-smelling odorants. Interestingly, the anosmics tended to generate an inodorous group containing AND, PEM, and orange. Thus, a non-verbal, semantic-free scaling method produced classifications consistent with those found when subjects reported odor qualities from a defined list of quality descriptors and retained the individual differences characteristic of osmic, allosmic and anosmic subjects.

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Subjective Hyposmia: Seven Cases

ALAN R. HIRSCH (Smell & Taste Treatment and Research Foundation) LINDA TALYA (University of Illinois Medical School, Chicago)

Seven patients presented complaining of olfactory impairment of at least 6 months duration. Despite this, none displayed objective olfactory impairment on Amoore's Unilateral Threshold Tests of PM-Carbinol. Other tests of olfactory threshold also displayed predominantly normal results, including Amoore's Unilateral Threshold Tests of PM-Carbinol (4 of 4), PE Phenol (3 of 4), Napthaline (4 of 4), Pyridine (1 of 4), Cineole (2 of 4), ISOB-ISOB (4 of 4), IA Acetate (2 of 4), PD Lactone (3 of 4), and CA Phenone (3 of 4). Possible factors in the etiology of this syndrome include test error, smell blindness, central hyposmia, episodic hyposmia, psychiatric disorder, and malingering. Awareness of potential underlying physiological and psychological factors and modification of testing procedures may help clinicians to identify these conditions.

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Psychosocial Aspects of Chemosensory Disorders. TAMARA J. KURTZ, THERESA L. WHITE, DANIEL B. KURTZ, ELIZABETH M. BELKNAP (Smell and Taste Disorders Clinic, SUNY Health Science Center, Syracuse, NY 13210).

In an effort to further describe the psychological impact of odors on the quality of life, 48 patients (32 women and 18 men) from the Smell and Taste Disorders Clinic were questioned concerning the reasons they had come to the clinic, and the psychosocial effects of their chemosensory disorders. Most patients came to the clinic seeking treatment for their disorders. Women were three times as likely to seek information about their disorders, and were three times more likely to report being worried about the possibility of a life-threatening disease. Patients reported a variety of life effects as a result of their disorders. The most common concerns were for safety (the inability to detect smoke, gas leaks, fire, and/or spoiled food) and a decline in the pleasure associated with eating. About one third of the patients reported that their chemosensory disorder resulted in a decrease in the quality of their lives, in that they were unable to experience the subtle, everyday pleasures associated with odors. Women were about three times more likely to express negative emotional reactions compared to men. Negative emotional reactions included depression, anxiety, frustration, loneliness, irritability, and a loss of self-esteem. However, the incidence of depression in both men and women, as measured by the Beck's depression inventory, was near population norms (Beck et al., Arch Gen Psychiat, 4:561-571. 1961) . On the other hand, the incidence of anxiety was quite high (31% with minimal to moderate anxiety measured on the Zung Anxiety Scale [Zung, Psychosomatics, 12(6):317-319, 1971]. Some women also reported a change in social and recreational activities (reluctance to dine out) as a result of their illness.

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The effect of capsaicin and pentanoic acid stimulation on the sensitivity of trigeminal neurons

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The perception of thermal, chemical, and mechanical irritants in the mouth is mediated by different types of neurons in the trigeminal nerve. These neurons, which are either single or multimodal, contribute to the overall perception of irritation in the mouth. The quality of the irritation depends upon the types of neurons activated and their relative sensitivities. We have investigated how the sensitivity to acidic stimuli can change with repeated exposure to chemical irritants. The responses of trigeminal neurons were determined for five different types of stimuli: cool (17-22 °C), cold (6-14 °C), warm/hot (39-45 °C), mechanical (pinching), and acid (150 mM pentanoic acid). Neurons were then repeatedly stimulated with 1 mM capsaicin or 150 mM pentanoic acid. Following this treatment, the test stimuli were presented again to study changes in both sensitivity and modality. This was followed by rinsing to determine recovery. Neurons, sensitive to acids and cool/cold but not to capsaicin, were desensitized to acidic stimuli by capsaicin. However their response to thermal stimuli was unaffected. Experiments which demonstrated capsaicin-induced extravasation in oral tissues suggest that vascular changes may be involved in the specific desensitization to acid stimuli. Treatment by repeated presentation of acid affected most of the recorded neurons. A variety of effects was observed across neurons: sensitization to acid, desensitization to acid, changes in thermal sensitivity, and changes in spontaneous activity. These results will be discussed in relation to coding mechanisms of irritation in the mouth.

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Tongue Adaptation Temperature Influences Lingual Nerve Responses to Thermal and Menthol Stimulation. ROBERT F. LUNDY JR. and ROBERT J. CONTRERAS (Florida State University, Department of Psychology, Tallahassee, FL., 32306-1051)

The present study investigated the effects of tongue adaptation temperature (35 °C vs 25 °C) on the neural responses of lingual nerve fibers to: (1) a 1 °C/s temperature decrease until reaching a plateau of 10 °C, and (2) menthol stimulation for 40 s. We studied the electrophysiological responses of 13 thermal-sensitive fibers in male Sprague-Dawley rats. In Phase 1, two groups of fibers were identified on the basis of response latency, peak sensitivity, and range of sensitivity after adaptation to 35 °C water. The response latencies of Group 1 fibers (n = 7) were ≤ 4 s or responding to a temperature decline ranging between 1 - 4 °C. Most Group 1 fibers (71%) continued responding (≥ 1.64 SD units above prestimulation rate) until reaching a temperature decrease of 6 - 9 °C and then returned to prestimulation rate. Thus, their range of sensitivity was between 34 - 23 °C. In contrast, Group 2 fibers (n = 6) responded to a temperature decline beginning between 5 - 8 °C. Group 2 fibers continued responding to temperature decreases of 10 - 15 °C. The temperature sensitivity of Group 2 fibers ranged between 30 - 12 °C. The mean peak response of Group 1 fibers was 28.0 ± 1.5 °C and that for Group 2 fibers was 23.5 ± 2.3 °C. Based on data from 4 of the 13 fibers, tongue adaptation to 25 °C water greatly attenuated the capacity of thermal-sensitive lingual fibers to respond to temperature decreases. In Phase 2, we examined the responses of lingual fibers to 0, 25, 50, and 75% dilution of a stock menthol concentration (1.28 mM) at 25 ° and 35 °C adaptation temperatures. Depending on the concentration and time of measurement, all menthol concentrations elicted significantly larger neural responses at 35 °C compared to 25 °C. Furthermore, only during 35 °C adaptation did responses to menthol stimulation persist during the ensuing 20-s after menthol off-set and water rinse on-set. Although an effective coolant, the degree of perceived cooling due to menthol in the oral cavity depends upon the temperature of the vehicle and the surface to which it is applied.

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Acetazolamide Inhibits Ethmoid Nerve Responses to Carbon Dioxide But Not to Other Irritants. W. L. SILVER and J. L. ERIKSEN (Wake Forest University, Winston-Salem, NC)

Nerve fibers in the ethmoid branch of the trigeminal nerve innervating the nasal cavity respond to a wide variety of chemical stimuli. Carbon dioxide is an irritant that stimulates ethmoid nerve fibers. Neural responses to carbon dioxide from the carotid body (Iturriaga et al., 1991, *Am. J. Physiol.* **261**: C565-C573) and the lingual branch of the trigeminal nerve innervating the oral cavity (Komai and Bryant, 1993, *Brain Res.* **612**: 122-129) have been shown to be dependent on the enzyme, carbonic anhydrase. Carbonic anhydrase catalyzes the hydration of carbon dioxide which is followed by the dissociation of H_2CO_3. In the present study we examined the effect of carbonic anhydrase on ethmoid nerve responses to carbon dioxide and other irritants. Male, Sprague Dawley rats weighing 350 - 400g were anesthetized with Urethane (1.0 g/kg, i.p.) The procedures for recording from the ethmoid nerve and for delivering the stimuli have been described previously (Silver et al., 1990, *Chem. Senses* **15**: 701-712). The olfactometer was modified so that carbon dioxide (25%, 50% and 100% in air) could be delivered to the rat's nares. Multiunit ethmoid nerve responses to carbon dioxide, propionic acid and amyl acetate were obtained. Acetazolamide (15 mg/kg) was then injected into the tail vein. Control rats were injected with the vehicle alone. Within 10 minutes of the acetazolamide injection, responses to carbon dioxide were eliminated while responses to propionic acid and amyl acetate were unaffected. No change in the response to carbon dioxide was seen in controls. These results demonstrate that carbonic anhydrase plays a role in the ethmoid nerve response to carbon dioxide but not to other chemical stimuli.

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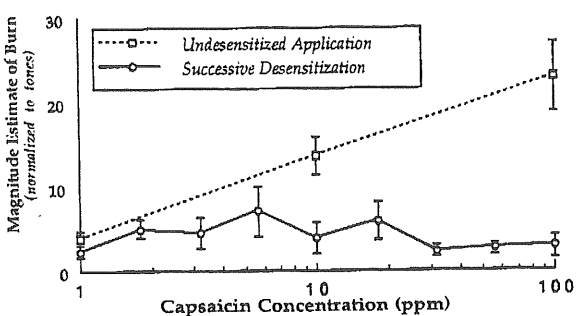
Successive Desensitization: a Low Pain/High Dose Technique for Oral Capsaicin Delivery. WOLFFE NADOOLMAN, VALERIE B. DUFFY, ANN M. BERGER, LINDA M. BARTOSHUK (Yale University, New Haven, CT.)

Capsaicin causes both excitation and desensitization of nociceptive neurons. The extent of desensitization has been previously shown to be related to the extent of excitation (pain). For therapeutic anesthetic applications, a technique allowing the delivery of a high dose—with associated high levels of pain desensitization—without the usual accompanying discomfort would be helpful.

A regimen of graduated solutions of capsaicin, ranging from 1ppm (3.3mM) to 100ppm (0.3mM) was applied to the tongues of 10 adults. The burning sensation associated with each application was allowed to fade before the next solution was applied. On average ($p<.002$), the subjects reported a burning sensation associated with the highest-strength solution (100ppm) that was 12% of the intensity they reported without this protocol.

Magnitude Estimate (±se) of the Burn of Capsaicin before and after Successive Desensitization



Successive desensitization could permit the clinical use of high topical capsaicin doses with only moderate associated subject discomfort.

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Principal Components Analysis of Astringency and Sourness Ratings of Acids, Alum and Mixtures. HARRY LAWLESS and CAROL CORRIGAN (Cornell University).

In two experiments, subjects rated the intensity of astringency, drying, roughing, puckering and sour sensations from citric, phosphoric, hydrochloric and malic acids over time (15 to 30 sec intervals for 4 min). In a third experiment, mixtures of alum with gallic acid and alum with citric acid were similarly rated as well as the single component stimuli (bitterness was also rated). Previous analyses showed patterns of response that were partially decorrelated for the various rating scales, supporting the idea that they are independent perceptual attributes and that astringency has several subqualities or separate perceptual components. In order to provide an additional mathematical test, ratings from the first or second time intervals (peak intensities) were submitted to principal components analysis. Unrotated solutions loaded all astringency and tactile variables on the first factor, and sourness on the second. Rotated (varimax) solutions separated drying, roughing and sourness on separate factors, with puckering and astringent loading heavily on the same factor in the first two studies. The rotated solution for the mixture study gave separate loadings for sourness, bitterness and puckering, with drying and roughing loading heavily on the first factor. In all original correlation matrices, puckering was more highly correlated with astringency than with sourness. These patterns suggest the following conclusions: 1) that the astringent acids elicit tactile responses different from their taste properties, 2) that puckering is more synonymous with astringency than with sourness, and 3) that there is some overlap in the perceptual effects of acids regarding astringency and its subqualities, althought there is also independent variance associated with each attribute.

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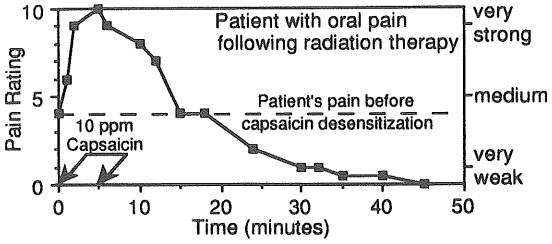
Capsaicin Desensitization Can Abolish Oral Pain. T. KARRER (International Flavors and Fragrances), L.M. BARTOSHUK (Yale University School of Medicine).

Capsaicin, the compound responsible for the burn of chili peppers, produces pain when first applied to the tongue but subsequently desensitizes chemical pain fibers. We have previously described desensitization to capsaicin in normal volunteers. Of particular importance, desensitization to a given concentration of capsaicin reduces that concentration to or near zero although higher concentrations still produce perceptible burn (albeit reduced in intensity). In patients whose oral pain is mediated by chemical pain fibers, desensitization to capsaicin should reduce the patient's pain.



Three patients who suffered oral pain in the absence of lesions, one with Epstein-Barr, one post-radiation therapy (see figure), one with pain of undetermined origin following surgery for an acoustic neuroma, were desensitized to 10 ppm capsaicin by swabbing a capsaicin solution on the painful area for at least 5 min. As the pain of capsaicin subsided, so did the patient's pain. In two cases, patient's pain was abolished. For the third patient, 10 ppm reduced but did not abolish his pain but 100 ppm abolished his pain. The patients' pain did not return to its original intensity for more than 24 hours.

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Prior experience with capsaicin alters ratings of its burn RICHARD J. STEVENSON and JOHN PRESCOTT CSIRO Sensory Research Centre, Sydney, Australia.

Though it has been recognised for some time that differential prior experience with an oral stimulus (especially capsaicin) can alter ratings of its intensity, there has been little empirical interest in this phenomena. To investigate this two matched groups of subjects were exposed to either high (16 p.p.m.) or low (1 p.p.m.) intensity chilli burns, at four day intervals (to discount desensitization), four times. On a probe trial (4 p.p.m.) four days following the last exposure, subjects previously exposed to high chilli burns rated the probe as significantly less intense than subjects exposed to low chilli burns. To examine whether this effect resulted from successive contrast or some more generalised feature of the exposure context, a second experiment was conducted. Again, two matched groups were formed and exposed to either an ascending or descending capsaicin series (0.25 - 16 p.p.m.), so that both groups 'generalised experience' was the same, but the trial preceding the probe was very different. This experiment revealed no significant difference between groups suggesting that the effect in the first experiment may have resulted from some generalised memory of the intensity of the preceding exposures, rather than a successive contrast with the last exposure trial.

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The Cross-Modal Relationship between Vision and Olfaction is Dimensional: Color Value Varies Inversely with Odor Intensity.
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Cross-modal correspondences between vision and audition have been well described, but correspondences between vision and olfaction have not. A previous experiment from our laboratory demonstrated that color hue, value and chroma vary with odor quality (Gilbert and Martin, 1992). This experiment investigates whether these color dimensions change systematically with odor intensity. Thirty-eight male and female subjects (29 ± 7 years) were screened from a total of 50 subjects for normal sense of smell, absence of active head cold, sinus infection or allergy, normal color vision using Ishihara test plates (1990) and ability to perform a scaling task. Five odorants, previously found to have strong cross-modal associations to particular hues, were presented at three concentration levels. Visual stimuli were all 1490 chips from the Munsell Book of Color (glossy) and 78 chips from the Supplemental 80-Hue Colors. They were presented under D75 and UV lights in a portable light box, with neutral gray interior, placed in a booth in an ASTM standard sensory laboratory. The 15 odor stimuli were presented in random order. Subjects indicated the single color chip that best matched each odor. The odors were presented in random order a second time and rated for intensity on a 15 cm line scale, anchored "no odor" and "strong odor" (physically referenced by pure diluent and 80% olibanum oil respectively). Repeated measures ANOVAs with odor and concentration as within-subject factors, and sex as a between-subject factor, showed that the color dimensions of value and chroma each varied significantly with odor (all $p < .001$). Intensity varied significantly with odor ($p < .001$) and, as expected, with concentration, and there was a significant interaction between the two ($p < .001$). No sex differences were found. Across odors, there was a significant negative correlation between value and intensity (Pearson's $r = -.27$, $p < .001$); stronger odors were associated with darker colors. Across subjects, correlations between value and perceived intensity of a given odor were generally negative, and were significant for cinnamic aldehyde ($r = -.33$, $p < .001$) and methyl anthranilate ($r = -.43$, $p < .001$). Strength of the correlation depended on range of perceived intensity. In summary, it appears that the cross-modal relationship between vision and olfaction is dimensional: color value varies inversely with odor intensity. This finding parallels the dimensional cross-modal relationship of vision and audition, in which brightness varies with loudness.

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The Effect of Oral Capsaicin on the Perceived Intensity and Detectability of Taste and Retronasal Odor. BARRY G. GREEN (Monell Chemical Senses Center).

The extent to which oral capsaicin influences the perception of flavor has been controversial. The amount of gustatory masking reported in previous studies has varied depending upon both the experimental paradigms that were used and the concentrations of capsaicin that were tested. In the single study that examined masking of retronasal olfactory perception by oral irritation, odor intensity was reduced approximately as much as taste intensity, which suggested the masking effect had a central rather than a peripheral origin [Lawless, et al., *Chem.Senses*, 10:579-589, 1985]. The original aim of the present study was to determine if capsaicin would affect the perception of a complex "flavor" that was composed of both a gustatory stimulus and a retronasal odor. In the first experiment subjects used the oral labeled magnitude scale to rate the flavor intensity of whole-mouth mixtures of sucrose and citral presented simultaneously with 0, 2 or 5 ppm capsaicin. No significant masking was found for either level of capsaicin. The absence of a clear masking effect at suprathreshold intensities raised the question of whether capsaicin might at least make it more difficult to *detect* a taste or an odor. In the second experiment subjects received separate solutions of sucrose or citral with or without 5-ppm capsaicin in a two-alternative, force-choice detection paradigm (modified up-down staircase). Adding capsaicin raised the thresholds for detecting sucrose [$F(1,10)=17.60$, $p<0.005$)] and citral [$F(1,10)=5.80$, $p<0.05$], albeit in both cases by less than 0.5 log units. Taken together, the absence of significant masking at suprathreshold levels, the relatively modest increases in threshold, and the occurrence of threshold shifts in olfaction as well as in taste, all support the hypothesis that oral capsaicin interferes with the perception of flavor primarily via a central, cognitive effect (e.g., distraction or confusion) rather than via a peripheral, sensory effect (e.g., disruption of transduction).

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Taste and smell function in HIV infection. Camilla S. Graham, Brevick G. Graham, John A. Bartlett, Alison E. Heald, and Susan S. Schiffman (Duke University).

The purpose of this study was to determine if losses in taste and smell function occur during progressive HIV infection. If alterations do occur, they could be a consequence of HIV itself, concomitant opportunistic infections or concurrent medical treatments. Taste and smell function was evaluated in 40 HIV-infected individuals and 40 healthy control subjects matched with the HIV-infected patients for age, sex, race, smoking behavior, and number of years of education. Chemosensory tests administered to subjects included taste detection thresholds for glutamic acid and quinine hydrochloride, smell detection thresholds for phenethyl alcohol and menthol, taste and smell memory tests, taste and smell discrimination tests, and taste and smell identification tasks. ANOVA yielded a significant effect of HIV status, with the experimental subjects showing significantly impaired performance compared with controls on the following measures: glutamic acid taste detection threshold, $F(1,64)=12.10$, $p<0.001$; menthol smell detection threshold, $F(1,64)=4.60$, $p<0.05$; and the smell recognition of differences task, $F(1,64)=5.79$, $p<0.05$. The losses in patients in the latest stage of HIV infection (stage C3) were even greater than in less severe stages of the disease. These results indicate that there are taste and smell losses in progressive HIV infection, although it can not be determined whether these losses are caused by the disease itself or related infections and treatments.

Spatial Taste Abnormalities After Head Trauma. A. MOTT*, W. MYERS, J. GENT+, M. BARWICK*. (Univ. of CT Health Center, Farmington, CT *School of Medicine; +John B. Pierce Laboratory, New Haven, CT.)

Taste loss after head trauma is reported to be less severe, less frequent and more reversible than trauma-related olfactory loss. Some reports suggest that bitter deficits may be more frequent and longer lasting than deficits to other taste qualities. The purpose of this database study is to verify the frequency, type and severity of taste loss after head trauma (HT). Subjects in the Connecticut Chemosensory Clinical Research Center (CCCRC) database were classified into 2 broad HT categories; no history of head trauma (*HT never*; n=354) and any history of head trauma (*HT ever* = transient loss of consciousness or disorientation; facial or skull fracture; head laceration; symptoms of post-traumatic syndrome; n=161). Subjects with *HT ever* were further classified into *HT etiology* (n=73) and *non-etiologic HT*. Subjects were defined as HT etiology if HT was judged to be the cause of a chemosensory disorder. Whole mouth taste for NaCl, sucrose, citric acid, and quinine HCl was measured by the CCCRC suprathreshold magnitude matching test. The CCCRC spatial taste test was used to assess seven discrete areas of taste (swallow; rt. and lt. palate, ant. tongue and post. tongue) for each taste quality. Results: *Whole mouth scores* were not significantly different for *HT ever* vs. *HT never* (two-tailed t-test), although there was a statistical trend toward decreased citric acid ($p=.07$) taste in the *HT ever* group. *Spatial taste:* Ageusic areas were more frequent in the *HT ever* group (chi-square) (citric acid, $p=3.8 \times 10^{-5}$; sucrose, $p=.03$; NaCl, $p=.05$; trend for quinine HCl, $p=.06$). *Non-etiologic HT* subjects showed significance for citric acid ($p=.01$) and a trend for quinine HCl (.06). *Etiologic HT* subjects showed the greatest number of ageusic areas (citric acid, $p=.0002$; sucrose, $p=.0002$; NaCl, $p=1.8 \times 10^{-5}$; quinine HCl NS). Differences in spatial intensity ratings for subjects with *HT ever* versus *HT never* were analyzed using repeated measures multivariate analysis of variance (MANOVA). Spatial intensity ratings were lower in *HT ever* subjects ($p<.0001$), although we did not see a differential effect of HT on taste quality or specific spatial areas. These analyses verify that despite preservation of whole mouth taste, subjects with even a remote history of HT show persistent, localized taste deficits when measured both by numbers of localized ageusic areas and spatial intensity ratings. Although the "controls" in these analyses have no history of HT, they are all CCCRC subjects with subjective chemosensory abnormalities and may therefore have measurable taste abnormalities. We are likely to show an even greater effect of HT on taste when normal controls are used for comparison. These data do not support previous reports that bitter taste is affected more than other taste qualities after head trauma.

This study was funded by NIDCD, PH51-DC00168.

Sweet Taste Depression using Two Different Inhibitors

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Effects of the inhibitors gymnemic acid and Na PMP on the sweetness of 5% sucrose, 5% glucose and 0.01% saccharin are examined by time-intensity analysis using the SMURF device and human subjects. Both inhibitors depressed both sweetness intensity and sweetness persistence in all three sweeteners but the % inhibition of sweetness persistence tended always to be less than % inhibition of sweetness intensity. Na PMP was effective at a much lower concentration (0.002 - 0.02%) than gymnemic acid (0.02 - 0.05%). However, both inhibitors showed an increasing trend of increasing inhibition with increasing concentration. Saccharin showed different effects from the sugars at certain concentrations of inhibitor and sweetener type caused significant differences in intensity of inhibited response but not persistence. These results support the separate identity of sweetness intensity and persistence mechanisms in chemoreception. When Na PMP is tested with the bitter sweet sugar mannose, there is no significant difference between the % inhibition of intensity and the % inhibition of persistence. However, Na PMP does not complicate the experiment by conferring a bitter taste like gymnemic acid. It is therefore an appropriate agent for comparing the bitter/sweet sugar with a glucose/quinine model mixture. In such systems the sweetness is depressed leaving the bitterness essentially unaffected.

Probabilistic Models for Sequential Taste Effects in Triadic Choice. **DANIEL M. ENNIS (Philip Morris Research Center, the Medical College of Virginia and the University of Illinois), SUSAN TEDJA, RYUICHI NONAKA AND MICHAEL O'MAHONY (University of California, Davis)**

Sequential effects and positional response bias are accounted for in new models for triadic choice. These models were applied to data on distilled water and dilute NaCl solutions using the triangular and 3-AFC methods with four subjects. The concept of a "conditional stimulus" is introduced to describe stimuli that are created partially by prior oral environmental effects. The effect of one or two prior stimuli on triadic choice was evaluated. The triad models used were based on a Thurstonian variant of Richardson's method of triads and a Thurstonian model for first choice among three possibly different stimuli. Maximum likelihood estimates of the scale values for conditional stimuli and bias parameters showed that it was necessary only to consider one prior stimulus. It was also shown that salt concentration differences are not the physical analog of the mental representations for the conditional stimuli. The results strongly suggest a water taste → salt taste continuum. The models discussed could be applied widely to triadic choice experiments in which sequential effects occur and extensions of them could be developed for other choice paradigms.

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A Taste Confusion Matrix. MARION E. FRANK, THOMAS P. HETTINGER, JANNEANE F. GENT* and LAWRENCE E. MARKS* (UConn Health Center, Farmington and *J.B. Pierce Laboratory, New Haven CT, USA)

H.N. Wright[1] described a method to assess the qualities and dimensions of olfactory perception; this method relies on the principle that similar substances are confused with one another in identification tasks. A confusion matrix for 10 substances was generated without training. We have applied this method to the taste system. Each of 10 subjects tasted 0.1M NaCl, 0.1M KCl, 0.1M Na glutamate (MSG), 0.1mM quinine.HCl, 3mM citric acid, 0.3M sucrose, 3mM aspartame, and NaCl-sucrose, acid-sucrose, and quinine-sucrose mixtures; then chose the name from a list: "salt, salt substitute, MSG, quinine, acid, sugar, artificial sweetener, salt-sugar, acid-sugar, quinine-sugar" in 10 consecutive replicates. The correct stimulus was identified most frequently but percent-correct ranged from 71% for acid and the acid-sucrose mixture to 37% for KCl (salt substitute). The pattern of errors was consistent. For example, sucrose was identified as sugar or artificial sweetener 97% of the time and MSG was identified as MSG or salt substitute 78% of the time. The acid-sucrose mixture was confused with quinine-sugar 18% of the time, reflecting a 14% acid-quinine confusion. The salt-sucrose mixture, identified correctly 70% of the time, was confused with sugar 12% of the time, reflecting a mixture interaction. Individual subjects varied in consistency (percent information transmitted) from 58% to 88%. The average amount of information transmitted (bits) was 2.4 for taste, which compares with reports of 2.5 for pitch and 3.1 for hue. Taste confusion matrices hold promise for studies of taste discrimination and for clinical evaluation of individual patients.

[1]Arch Otolaryngol Head Neck Surg 113:163-8, 1987.
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A Psychophysical and Anatomical Study of the Development of the Sense of Taste in Adults and Children

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Little is known about when the sense of taste becomes adult in function in humans, The behavioral data available are mostly from preferences studies and there are no definitive anatomical studies of peripheral development. Accordingly, in the present study a two-alternative forced choice psychophysical procedure was used to determine the sensitivity of 12 localised areas of the anterior tongue of adults and 8yr old males to 0.2125M sucrose or water delivered on a filter paper. In addition, papillae in each area were counted. The results indicated that children were more sensitive than adults to the sweetener at 8 of the areas, that both groups had similar numbers of papillae at 8 areas, whilst at 3 of the areas where children exhibited a higher sensitivity, children also had more papillae. Since 8 yr old males are less sensitive than adults to sucrose when it is given as a whole-mouth stimulus (James and Laing, 1993), it is proposed that (i) the childrens gustatory system may be unable to integrate the information available at the tongue as efficiently as adults, or that the threshold and stimulus-response functions of receptor cells are different in the two groups, and (ii) the human gustatory system is not fully developed in 8 yr old males.

C James and D G Laing, 2nd Intern. Multidisciplinary Conf. on Food Choice, Adelaide, Australia, 1993.

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Detection of Additive, Cross-Quality Taste Mixtures. JOSEPH C. STEVENS and JULIANNE M.. HOFFMAN (John B. Pierce Laboratory)

Ability to detect four substances (sucrose, sodium chloride, citric acid, and quinine hydrochloride), alone and in mixture with one, two, or all three of the others, came under study in eleven young subjects. On each trial the forced-choice task was to distinguish between a water sample and a sample containing a mixture (or one of its components). This kind of mixture revealed striking, highly reliable additivity, in that the threshold of each component in mixture was always lower than the threshold of each component measured alone. Of the six binary mixtures, four displayed hyper-additivity (components in mixture averaged less than half of each alone), and two hypo-additivity (compounds in mixture averaged slightly more than half each alone); of the four ternary mixtures three displayed hyper-additivity (compounds in mixture averaged less than one-third alone) and one hypo-additivity; the quaternary mixture displayed simple additivity (compounds in mixture averaged one-fourth those alone). In contrast to this liberal additivity at threshold, supra-threshold mixtures assessed by scaling are typically hypo-additive. (Olfactory mixtures at and above threshold have been shown to behave much the same way, ACHEMS Abstract #176, 1993.) Sucrose plays a dominant role in both kinds of mixture; mixtures with sucrose consistently gave the most hyper-additivity. Whatever the underlying mechanism may be, substances of different taste qualities prove easier to detect in concert than alone, as if the important thing is simply to distinguish a taste from a non-taste. However, as we reported earlier (ACHEMS, Abstract #11, 1992) ability to detect any particular quality is disturbed by the presence of another; threshold for the target quality rises with the concentration of the other quality. One must distinguish between mixtures that are additive (facilitatory) and those that are subtractive (inhibitory). Supported by NIH Grant AG04287.

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Amiloride suppresses the saltiness of LiCl but not KCl in humans.

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Several psychophysical studies have demonstrated that many humans experience a suppression of the saltiness of NaCl in response to stimulation with amiloride, a sodium channel blocker. In this study, the salty tasting compounds of LiCl and KCl were tested. Five subjects gave magnitude estimations of the saltiness of LiCl (0.10M, 0.30M, 0.50M) and KCl (0.10M, 0.20M, 0.30M). They dipped the anterior portion of the tongue into 10 ml solutions of LiCl and KCl mixed with either water, quinine (used as a control for the bitterness of amiloride) or amiloride (500 M) and gave magnitude estimates of the saltiness of these compounds. The mechanism of transduction of the saltiness of LiCl, but not KCl, is proposed occur through amiloride-sensitive channels. The results indicated that, in all subjects, the saltiness of LiCl was reduced (by approximately 52% across subjects) with exposure to amiloride. One subject showed some suppression in KCl ratings and four subjects showed a small increase (approximately 18% across subjects) in the saltiness of KCl. As in other studies, individual differences were seen in this suppression; some subjects showed a more pronounced suppression than others.

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Effects of Amiloride on Human Taste Perception: Implications for Na^+ Receptor Mechanisms. CORINNE A. OSSEBAARD and DAVID V. SMITH (University of Cincinnati College of Medicine)

The sodium channel blocker amiloride has been used extensively to explore the mechanisms of sodium salt taste. Reduced responses to NaCl after amiloride treatment are seen in both chorda tympani fibers and taste receptor cells. Human psychophysical experiments show either a moderate reduction in the saltiness of NaCl or no reduction at all. These studies suggest an amiloride insensitive component (AIC) in the response to NaCl and the existence of a non-amiloride sensitive sodium receptor mechanism, which may be larger in humans. Ye *et al.* (1993) propose that there are sodium channels on the basolateral as well as on the apical taste receptor membrane. They suggest that the accessibility of Na^+ to these basolateral channels depends on the size of the anion. Sodium salts with large anions, like Na-gluconate, only have access to the apical membrane, whereas sodium salts with small anions, like NaCl, have access to both membranes. Since amiloride only reaches the apical membrane, the AIC will be larger for NaCl than for Na-gluconate. In the present study, this hypothesis was tested for human taste perception. Five different concentrations of NaCl, Na-gluconate and KCl were used as test stimuli after adaptation to distilled water or to 50 μM amiloride. Single concentrations of sucrose, citric acid, QHCl and amiloride were also tested. Subjects estimated total intensity and divided this estimate into saltiness, sweetness, sourness, bitterness and other tastes. Stimuli were applied by gravity flow to the dorsal part of the tongue, at a rate of 6 ml/sec. Results show that amiloride reduced the perceived intensity of both NaCl and Na-gluconate, but had no effect on KCl, citric acid or sucrose. The bitterness of QHCl was somewhat reduced, presumably via cross adaptation. Interestingly, the largest effect of amiloride was on the reported *sourness* of NaCl (69% reduction at 1 M) and Na-gluconate (88% at 1 M); the response to the larger anion was more suppressed, as predicted. Supported in part by NIDCD Grant DC00353 to D.V.S.

Receptor Neuron Responses to Pheromone Compounds and Formate Analogues in the Noctuid Moth *Heliothis virescens*.
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Two principal pheromone components, *cis*-11-hexadecenal (Z11-16:AL) and *cis*-9-tetradecenal (Z9-14:AL), elicit attraction and sexual behavior in the male moth *Heliothis virescens*. It is shown in the field that one aldehyde, Z11-16:AL, can be substituted by the formate analogue *cis*-9-tetradecen-1-ol formate (Z9-14:FO), whereas the other aldehyde, Z9-14:AL, can not be substituted by the corresponding *cis*-7-dodecen-1-ol formate (Z7-12:FO). It was therefor interesting to study the structure-activity relationships of the aldehydes and the formate analogues on the receptor neurons that receive information from the two principal pheromone components. Three types of receptor neurons were identified on the male antenna, each tuned to one of three biological signals; the pheromone components Z11-16:AL and Z9-14:AL and *cis*-11-hexadecenylacetate (Z11-16:AC) which is assumed to interrupt the pheromone attraction either as an intra- or an interspecific signal. The receptor neurons tuned to Z11-16:AL responded almost as strongly to the pheromone mimic Z9-14:FO as to its key compound. The neurons tuned to Z9-14:AL, however, were considerable less sensitive to the corresponding formate, Z7-12:FO (dose-respons curve shift of 3 log units to the right). This suggests that different parts of the two pheromone molecules dominate in the interaction with the two types of receptors. Furthermore, the third type of receptor neurons, tuned to Z11-16:AC, also responded to Z7-12:FO, with a dose-respons curve shift about 2 log units to the right. Altogether, these results explain why one formate (Z9-14:FO) can substitute the pheromone component Z11-16:AL, whereas the other pheromone aldehyde can not be substituted by the corresponding formate. It would not be possible to increase the amount of Z7-12:FO in order to simulate the effect of Z9-14:AL, since this would result in activation also of the receptor neurons tuned to Z11-16:AC which interrupt the pheromone attraction.

Projection Patterns of Single Pheromone Receptor Neurons in the Antennal lobe of the Male Tobacco Budworm Moth, Heliothis virescens.
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The antennae of the male tobacco budworm moth possess numerous olfactory sensilla, s. trichodea, the majority of which contain pheromone specific receptor neurons (RNs). The axons of the RNs enters the antennal lobe (AL), where the pheromone specific RNs form synapses with AL interneurons in the male specific macroglomerular complex (MGC). The female produced pheromone blend contains two principal components, (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-9-tetradecenal (Z9-14:Ald), which are sufficient to elicit the male sexual behavior. Three types of RNs are identified on the male antennae. Two types are tuned to each of the principal pheromones and the third is tuned to (Z)-11-hexadeneyl acetate (Z11-16:OAc) assumed to mediate interruption either intra- or interspecifically. In the present study, we have examined the morphological features of the MGC and the projection patterns of single pheromone RNs in the antennal lobes, using a combined electrophysiological and morphological technique. Single, physiologically identified RNs were marked with cobalt lysine during stimulation with the specific pheromone. It was found that the MGC consists of four major subunits. One large unit is situated at the entrance of the antennal nerve. In a frontal view, the three smaller subunits are all located medially of the larger unit in a dorsal, medio-lateral and ventral position respectively. All successfully stained neurons arborized exclusively within the MGC. Interestingly, the RNs tuned to Z11-16:Ald and Z9-14:Ald showed similiar projection patterns, with arborizations restricted within the large MGC unit. The RNs tuned to the acetate, however, all arborized in a different part of the MGC, the medio-lateral subunit. Thus, the MGC subdivisions seems to be functional units, where information from the pheromones and the acetate is processed in different parts of the MGC.

Comparative Analysis of Central Pheromone Information-Processing Circuits in Two Closely-Related Sympatric Noctuid Moths.
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The macroglomerular complex (MGC) in the antennal lobe of male heliothine moths receives convergent input from several chemically-identified olfactory pathways involved in the discrimination and location of potential mating partners. The MGC is made up of distinct subdivisions, and each subdivision is functionally and anatomically equivalent to a glomerulus, the basic subunit of odor-processing neuropil found in diverse animal species. The different glomeruli that make up the male-specific MGC are, furthermore, heterogeneous processing units, and this diversity is reflected in the different response types revealed by intracellular recording and staining of MGC interneurons. In the male tobacco budworm moth, *Heliothis virescens*, the range of physiological response types in MGC-output neurons reflects the different chemical components in the female sex-pheromone blend of this species. Out of a total sample of 32 MGC neurons examined in detail, most (85%) responded, but to varying degrees, to the principal component of the blend, (*Z*)-11-hexadecenal. Six neurons were activated solely by this component, whereas 8 neurons also responded to higher concentrations of the second essential component, (*Z*)-9-tetradecenal. In 14 neurons, the excitatory responses to each of these 2 components delivered at the same concentration were nearly indistinguishable, suggesting convergent input from both receptor pathways. One additional neuron responded in a unique fashion to the blend of the 2 pheromone components, indicating that at least some MGC neurons can function as "blend detectors". Curiously, none of the neurons thus far encountered responded selectively to (*Z*)-9-tetradecenal, in sharp contrast to some MGC neurons in a closely-related sympatric species *Helicoverpa* (formerly *Heliothis*) *zea*. These findings, along with details from laser-confocal microscopy of physiologically-identified neurons, suggest important differences in the functional organization of the MGC in these 2 closely-related species. They also illustrate several strategies by which information about a naturally-occurring mixture of odorants such as a pheromone blend can be represented in the brain.

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Giant 5HT-IR Neurons and Accessory Lobes in Crayfish Brain: Their Possible Role in Olfaction

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Neurobiotin fills of a pair of very large 5HT-IR neurons (dorsal giant neurons, or DGNs) on each side of the brain in the crayfish *Cherax*, indicate that each neuron terminates in all of the approximately 17,000 glomeruli in each accessory lobe (AL) ipsilateral to it and also in all the ipsilateral olfactory lobe (OL) glomeruli. The ALs receive no projections from primary afferent fibers, nor any branches of motorneurons but are connected across the brain by the deutocerebral commissure (DC), the axons of which terminate in the glomeruli of the ALs. Electrical stimulation of the olfactory afferents hyperpolarizes the DGNs, whereas electrical stimulation of the DC depolarizes them, leading to the conclusion that the excitatory input to the DGNs is through their terminals in the ALs and that the output of the DGNs is in the OLs (Sandeman and Sandeman 1994, in press). We have now found that some neurons in the DC respond to changes in light intensity and others to electrical stimulation of the second antennae. Filling DC neurons with neurobiotin revealed that all ended bilaterally in relatively few glomeruli in the ALs on each side of the brain. No two DC neurons end in the same glomerulus, but all share glomeruli with the DGNs. The AL may therefore be a site where selected non-olfactory afferents are combined with olfactory information to modulate processes in the OL via the DGN.

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Chemical Neuroanatomy of Central Inputs to the Main Olfactory Bulb in Vertebrates.

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The central nervous system controls and adjusts incoming flow and processing of afferent signals, and such control occurs at different levels of a sensory pathway. The main olfactory bulb (OB) is the first structure in the olfactory pathway which is influenced by central processes. The basic anatomical and neurochemical characteristics of central inputs to the OB reviewed here are follows: (i) the OB receives multiple inputs from olfactory and non-olfactory structures of the forebrain and brainstem; (ii) a majority of neurons projecting to the OB are placed in the structures of the primary olfactory cortex and send their axons unilaterally, whereas some non-olfactory structures have bilateral projections; (iii) central fibers terminate on intrinsic bulbar neurons which are basically inhibitory; (iv) there is no clear correspondence between the origin of an axon in the brain and the site of its termination within the OB; (v) bulbopetal fibers from all brain sources project to granule cells, but axons from non-olfactory structures also reach elements in the glomerular layer; (vi) central influences are mediated by a series of neuroactive substances, including "classical" transmitters and neuropeptides; (vii) the OB receives both excitatory and inhibitory inputs which are likely tonic in character; (viii) bulbopetal neurons, located within the same brain structure, often belong to several cell populations differing in their transmitter specificities. The mode of central influences on the OB is thought to be dependent on the origin of bulbopetal fibers, the sites of their termination and the neurotransmitter used. Our recent findings, as well as specific features of central innervation of the OB in different vertebrate species, will be discussed.

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Evidence for Modulation of Amino Acid Receptors by Endogenous Zinc and Copper. PAUL Q. TROMBLEY and GORDON M. SHEPHERD (Section of Neurobiology, Yale Medical School, New Haven, CT 06501)

Information processing in the glomerular region of the olfactory bulb involves complex interactions between glutamate, GABA, and possibly glycine acting at multiple types of amino acid receptors. Olfactory nerve terminals contain a high concentration of zinc (Zn), a transition metal that has been shown to modulate amino acid receptors including NMDA receptors and some forms of $GABA_A$ receptors (GABAR). The olfactory bulb also contains a high concentration of copper (Cu) which, like Zn, recently has been shown to modulate GABARs. The similarity between the effects of Zn and Cu on GABARs led us to hypothesize that Cu may also modulate NMDA receptors. Similarly, because previously we have demonstrated that Zn could modulate GABARs but not glycine receptors (GlyR) we further hypothesize that GlyRs are insensitive to the neuromodulatory action of Cu. Olfactory bulb neurons from E18-P3 rat pups were grown in primary culture and examined using whole cell voltage clamp recording techniques. All olfactory bulb neurons tested responded to flow pipe application of NMDA (100 μM), GABA (30 μM), or glycine (100 μM). In all neurons examined (n=36) the current evoked by NMDA or by GABA but not by glycine was reversibly blocked by either Zn or Cu. A similar concentration of Zn or Cu (IC_{50} ~30 μM) was necessary to block 1/2 of the current evoked by GABA or by NMDA. The effects of Zn or Cu were not voltage dependent; either ion could block GABAR mediated Cl^- currents or NMDA receptor mediated non-specific cation currents at negative or positive membrane potentials. The lack of voltage dependence and the selectivity between GABARs and GlyRs suggests that Zn and Cu act at a specific neuromodulatory site rather than as an open channel blocker or by a screening effect. In other brain regions it has been demonstrated that Zn and Cu are released from nerve terminals by depolarization. The high concentrations of Zn and Cu in the olfactory bulb may suggest that these ions play a role in odor information processing through activity-dependent modulation of amino acid mediated synaptic transmission.

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Anatomical changes in the lateral antennule of the American lobster, *Homarus americanus*, during larval development

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Although much is known about adult lobster chemoreception, little is known about chemoreception in lobster larvae. During post-embryonic development, there are one prelarval and three larval stages, before the metamorphosis and subsequent settlement of the fourth post-larval stage. Lobster larvae are planktonic and each stage is morphologically distinct. The planktonic environment and diet is different than the benthic environment and diet of post-larval and adults lobsters. One may expect that the spectrum of chemical stimuli which are relevant to larvae may be different from that of the adult lobster. Before physiological studies on tuning can be initiated, detailed anatomical knowledge is necessary. The emphasis of the current study is to examine the development of the lateral antennule in larvae and to ascertain whether this organ possesses sensory neurons. The stage I lobster larva possesses a single giant sensillum at the distal end of the lateral antennule. It is unknown whether this sensillum performs a sensory function. Not until the larva molts into a stage II larva are the typical chemosensory aesthetasc hairs present. Unfixed larval lateral antennulae were observed using Nomarski DIC microscopy. Lobster larvae were molt-staged to determine whether the larvae were postmolt, intermolt, or premolt . During early premolt (stage Do), the formation of putative sensory fibers may be observed at the distal region of the antennule. The location of these fibers corresponds to the location of the aesthetasc sensilla of the stage II larvae. In late premolt (D2-3) stage I, the aethetasc hairs are apparent in a cluster at the base of the giant sensillum. Because the aesthetasc hairs develop from the fibers found at the base of the giant sensillum, it is possible that the giant sensillum is a protoaesthetasc sensillum and may also be chemosensory.

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Morphological and Physiological Transformations in the Olfactory Sensilla of Blue Crabs Acclimated to Low Salinity. RICHARD A. GLEESON[1], MICHELE WHEATLY[2], LORRAINE M. MCDOWELL[3], CARL L. REIBER[2], HENRY C. ALDRICH[3]. ([1]The Whitney Lab., [2]Dept. of Zoology, and [3]Dept. of Microbiology and Cell Science, University of Florida)

The olfactory sensilla (aesthetascs) of the blue crab are arranged in a dense tuft located on the outer flagellum of the antennule. Each aesthetasc contains the dendritic processes of from 40 to 160 chemosensory neurons. For much of their length, these processes are separated from the external environment by a thin cuticle that is permeable to odor molecules. Because the euryhaline blue crab ranges from seawater to freshwater, these olfactory dendrites can be exposed to variable osmotic/ionic conditions. In this study, the aesthetascs of blue crabs collected from freshwater environments were examined both morphologically and physiologically. Transmission electron microscopy revealed a marked reduction (relative to seawater acclimated animals) in the dendritic membrane exposed to the external environment; a characteristic commonly found in aesthetascs of crustaceans living exclusively in freshwater. This morphological change was reflected by a concomitant decrease in the intensity of responses to chemical stimulation of the aesthetascs as measured neurophysiologically. Experiments with extracellular tracers demonstrated a continuity between the hemolymph and sensillar lymph; these studies revealed a net efflux of sodium into the sensillar lymph. This sodium efflux, together with a reduced exposure of the dendritic membranes to the external environment, may represent important mechanisms for maintaining chemosensory function at low salinities.

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The Presence Of PDGFRß In The Adult And Young Mouse Olfactory System. WOOCHAN JANG and JOEL MARUNIAK (Division of Biological Sciences, University of MIssouri-Columbia, Columbia, MO 65211)

We have assessed the cellular localization of platelet derived growth factor ß type receptor (PDGFRß) in the mouse olfactory receptor cells (ORCs) and the olfactory bulb. With a polyclonal antibody, in the adult olfactory epithelium the knobs and the perinuclear region of the cell body of ORCs showed the positive PDGFRß immunoreactivity. The neuronal expression of PDGFRß is consistent with previous reports (Smits *et al.*, PNAS, 88, 8159-8163, 1991). In the adult olfactory bulbs, the external plexiform layer and the subependymal layer appeared to have PDGFRß. The presence of PDGFRß in the subependymal layer suggests possible roles of PDGF and its receptor in the neurogenesis and cell proliferation in this region. In the olfactory bulbs of young animals (P5), the mitral/tufted cell bodies showed the intense PDGFRß immunoreactivity suggesting different roles in the developing olfactory system. Since mitral cells undergo substantial maturation after birth, PDGF may play a role in their development. Immunoreactivity in the developing mitral cells decreased at about P10. The presence of the protein was confirmed by Western blot analysis. In addition, Northern blots revealed the expression of the PDGFRß message in the olfactory bulbs. The specific functional role of PDGF and its receptor during development and during neuronal turnover in normal and traumatic conditions (*e.g.*, neuronal lesion, bulbectomy or naris closure) is being investigated.

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The Horizontal Basal Cells of the Olfactory Epithelium. ERIC H. HOLBROOK, KAREN E. MIELESZKO SZUMOWSKI AND JAMES E. SCHWOB (Department of Anatomy and Cell Biology, and Chemosensory Disorders Group, SUNY Health Science Center, Syracuse).

The olfactory epithelium contains two categories of basal cells, horizontal and globose, neither of which is fully characterized. We have undertaken an immunochemical, electron microscopic and developmental analysis of the horizontal basal cell population, in order to delimit its potential biological role(s) in the epithelium. Immunochemical phenotype: We find that the HBC's of the olfactory epithelium share many characteristics with the basal cells in respiratory epithelium. For example, as others have shown before, the HBC's contain keratin-like immunoreactive material. We have used subunit specific monoclonal antibodies, whose specificity was verified using 2-dimensional immunoblots of epithelial homogenates, to demonstrate that the HBC's and the repiratory basal cells contain cytokeratins (CK) 5 and 14. Like respiratory basal cells, the HBC's also express a carbohydrate moiety to which *Griffonia* lectin, I-β_4, binds. Moreover, the HBC's stain with three different antibodies against the EGF-receptor (EGF-R), and with a monoclonal antibody that binds to phosphotyrosine; they do not stain with antibodies to the FGF receptor or the TGF-β-receptor. Interestingly, EGF-like immunoreactive material is prominent in the basal lamina underlying the HBC's. MeBr-induced injury to the epithelium causes HBC's to divide, and pile-up in some areas of the epithelium. Simple columnar cells, which do not have the phenotype of supporting cells, appear at the same time. Similar changes are observed with infectious rhinitis. Ultrastructual phenotype: The HBC's form a nearly continuous sheet of cells apposed to the basal lamina; however, the processes of supporting cells extend to the basal lamina and interrupt the HBC sheet. Before exiting the epithelium, virtually all olfactory axons traverse tunnels formed by the HBC's arching over the basal lamina. The intimacy of the arrangement between HBC's and olfactory axons suggests that signals may pass from axon to HBC's or vice-versa. Developmental appearance: Cells with the characteristics of HBC's do not become evident in the olfactory epithelium until late in embryonic development. At E19, basal cells expressing CK 14 and the EGF-R are prominent in the respiratory epithelium lining the anterior and ventral part of the nasal cavity. However, in olfactory epithelium, CK 14(+) and EGF-R (+) HBC's are found only in those areas that are adjacent to the respiratory epithelium and are lacking in the posterior turbinates and dorsal meatus. At later time points they appear in these dorsal and posterior areas. The developmental pattern suggests, but does not prove, that the HBC's migrate into olfactory epithelium from respiratory epithelium. We would suggest the following hypotheses: first, that HBC's respond to epithelial injury by undergoing mitosis, altering their phenotype and giving rise to the simple columnar cells; second, that EGF may be involved in this response.
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Long Term Survival of Olfactory Mucosa Transplants in Rat Brain. MORRISON, EDWARD E., PhD (Auburn University)
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It is well known that olfactory receptor neurons have a number of unique characteristics that separates it from other neurons in the vertebrate nervous system. Previously, we have demonstrated that these unusual characteristics persist even when olfactory mucosa is removed from the nasal cavity and placed in ectopic neural environments. In the present study we have examined the long term survival of olfactory mucosa transplants. Neonatal (P2-8) Sprague-Dawley rats served as host and donor animals. Olfactory mucosa (donor tissue) was dissected from nasal septum, cut into fragments 2-4 mm^2 and placed within parietal cortex of littermates (host animals). Host animals were examined 280-370 days post transplantation with light and electron microscopy. At these long term survivals there was no obvious host glial barrier and transplants blended with host brain tissue. Olfactory neuroepithelium was low, had identifiable neural elements, some reaching biochemical (OMP positive) and morphological (dendrites bearing cilia) maturity. Some regions lacked a basal lamina and underlying lamina propria and cells migrated from transplant tissue into the host brain parenchyma. Axons originating from transplant elements fasciculated into bundles, grew into and mingled with host brain tissue. Olfactory axons within host tissue formed axon terminals which contained mostly clear type synaptic vesicles. Spurious asymmetric synaptic complexes between transplant axons and host neurons were observed. These results indicate that olfactory mucosa transplant had long term survival within the rat brain, had the capacity to grow axon processes into host brain and form synaptic connections.

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A Comparison of PGP- and Calbindin-like Immunoreactivity in the Developing Human Nasal Epithelium

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There is a difference in the distribution of protein gene product 9.5 (PGP) and calcium binding protein D-28$_k$ (calbindin) immunoreactivity within mammalian nasal epithelium. Whereas PGP may be expressed in all[1] olfactory receptor neurons (ORNs), calbindin appears to be expressed within a subset of fetal ORNs, with diminishing expression during post-natal development[2]. It recently has been reported that human ORNs express PGP immunoreactivity[3] and that rat[4] and human[5] putative olfactory microvillar cells contain calbindin. There are no reports on whether these proteins are found also in the adjacent respiratory epithelium. In order to compare the distribution of immunoreactivity to these proteins between developing human olfactory and respiratory epithelia, we obtained human fetal and newborn tissues. Significant differences were observed. In the fetal and newborn olfactory epithelium (OE), virtually all ORNs were PGP 9.5-like immunoreactive (-LI). Only subsets of fetal ORNs were calbindin-LI. In the newborn, a small number of scattered calbindin-LI ORNs were seen. There was a marked contrast in labeling characteristics within the respiratory epithelium (RE). Unlike the OE, only a small number of PGP-LI epithelial cells were seen in the RE. PGP-LI beaded fibers were seen throughout the lamina propria; some penetrated into the mucosa. Also in contrast to labeling in the OE, there was an increase in the number of calbindin-LI epithelial cells in the RE during development. These data demonstrate a pattern of immunoreactivity consistent with the literature. Furthermore, the PGP-LI and CA-LI cells in the developing RE may constitute a subset of cells with biochemical characteristics that are shared by neurons and neuroendocrine cells. A few of these cells were also observed in the OE and had a morphological similarity to the microvillar cell. These issues will be discussed in terms of potential relevance to development.

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Glutathione distribution in the olfactory mucosa of rainbow trout following olfactory axotomy and copper toxicity.

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The tripeptide glutathione (GSH) and the conjugating enzyme GSH S-transferase (GST) protect tissues from the toxic effects of deleterious substances. In the olfactory mucosa GSH and GST may provide protection during events associated with ORC degeneration. We have previously used monobromobimane to identify a subpopulation of olfactory receptor cells (ORC) and melanophores with intense GSH staining in the olfactory mucosa of untreated rainbow trout (Starcevic *et al.* 1993, *Chem. Senses* 8:57-65). In this study, we have localized GST immunoreactivity in the apical cytoplasm of the olfactory epithelium and in olfactory nerve fascicles. ORC degeneration was induced either by olfactory axotomy or by exposing trout to copper sulfate. Before ORC death, GSH staining increased in the supranuclear region of the olfactory epithelium and in olfactory nerve fascicles, and decreased in melanophores adjacent to nerve fascicles. GST labeling remained intense in the apical olfactory epithelium and in olfactory nerve fascicles. Our results suggest that prior to ORC death, GSH is transported from melanophores to olfactory nerve fascicles and to the surface of the olfactory epithelium. Neuroprotection by GSH and GST may be occurring prior to ORC degeneration.

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Olfactory Responsiveness To Amino Acids And Sex Pheromones Return At Different Rates After Olfactory Nerve Axotomy In Goldfish.

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P. W. SORENSEN (Dept. of Fisheries & Wildlife, Univ. of Minnesota)

Recent studies of olfactory regeneration in goldfish (Zippel et al. 1993) demonstrate that ciliated olfactory receptor cells re-appear before microvillar receptor cells following olfactory nerve axotomy and that they are likely responsible for sensitivity to amino acids because regenerating epithelia never lose their sensitivity to these stimuli. This finding left open the question of what microvillar receptor cells detect. The present study addressed this question by simultaneously monitoring the return of behavioral and electrophysiological responsiveness to sex pheromones and food odors (which contained amino acids) in male goldfish following olfactory nerve transection. Using electro-olfactogram (EOG) recording we found that peripheral olfactory sensitivity to 10^{-3} M amino acids (L-Ala, L-Arg, L-Lys, L-Ser) and pheromones (derivatives of 17α, 20β-dihydroxyprogesterone and prostaglandin $F_{2\alpha}$) persisted for 3 days following axotomy (Post-Op) at which time sensitivity to the pheromones totally disappeared and sensitivity to amino acids dropped but did not disappear. EOG responsiveness to amino acids started increasing 2 weeks Post-Op at which time behavioral responsiveness to food odors also returned. In contrast, EOG sensitivity to sex pheromones did not re-appear until approximately 5 weeks Post-Op at which time behavioral responsiveness to spawning females releasing pheromones also returned. Although these data support the possibility that more slowly regenerating microvillar cells are responsible for responsiveness to sex pheromones, proof of this awaits histological confirmation, a process presently underway.

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Phenotypic Plasticity of Cells Dissociated from Embryonic Olfactory and Vomeronasal Organs.

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GRAZIADEI, P. P. C. (Florida State University)

The olfactory placodes and the developing olfactory and vomeronasal organs are able to differentiate according to their original developmental program when heterotopically grafted in a developing (Magrassi and Graziadei, 1985) or mature brain (Graziadei and Monti Graziadei, 1984). We were interested to study if the determinative ability shown by the organ primordia was maintained by their dissociated cells. To answer this question, we transplanted about 2 X 10^4 cells obtained from the olfactory and vomeronasal organs primordia of E12 rat embryos into the developing cortex, basal ganglia, brain stem, cerebellum, and ventricular system of E15 rat embryos. The cells were doubly labelled in vivo by bromodeoxyuridine injection to the donor mother, and in vitro by DiI after dissociation. The injected fetuses were sacrificed both prenatally (E21) and postnatally (P15,P30). The labelling of the cells allowed us to follow the fate of the cells after grafting. Grafted cells and their descendants were found dispersed in the CNS even at considerable distance from the injection site. Topological analysis, morphological observation of cellular profiles when outlined in the entirety by DiI and immunocytochemical staining with antibodies directed against GFAP, NSE, and NFs allowed us to study the fate of the engrafted cells. We have observed that cells dissociated from the developing olfactory and vomeronasal organs may, when transplanted into the fetal CNS, give rise to neurons and glia with a central phenotype. These findings suggest a remarkable plasticity of the olfactory matrix.

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Age- and Gender-Related Differences in the Expression of Glutathione *S*-Transferases in Human Olfactory Mucosa.

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The expression of Alpha, Mu, and Pi classes of glutathione *S*-transferases (GSTs) in human olfactory mucosa was investigated using immuno-cytochemical methods. Nasal mucosa (NM) was obtained at autopsy from 23 subjects of both genders including 2 fetuses (16 and 26 weeks of gestation), 2 young (0.2 and 24 yr.), 5 middle-aged (39-59 yr.), and 14 older (63-90 yr.) subjects, including 4 with Alzheimer's disease (AD). Olfactory mucosa was identified unequivocally by the presence of olfactory marker protein-immunoreactive receptor neurons in the epithelium. In the olfactory mucosa, GST Alpha was expressed predominantly in the acinar cells of the Bowman's glands (BGs), and to a lesser extent, in sustentacular cells (SCs). GST Pi was localized only in SCs. No detectable GST Mu immunoreactivity was observed. During ontogeny, GST Pi was first detected in 16 week fetus. GST Alpha was not expressed in BGs and SCs of prenatal stages; it was first observed in 0.2 year old subject. The intensity of immunoreactivity increased with age in young and middle-aged subjects. An age-related decrement in the intensity of immunoreactivity was observed in subjects over 60 years of age when compared with that in subjects under 60 years of age. Comparison of near-age-matched subjects of both genders revealed higher GST Alpha immunoreactivity in tissues from females than males. This was particularly evident in BGs. No apparent differences were observed in the NM of subjects with AD. These results suggest that GSTs, components of phase II biotransformation, are actively involved in olfactory perireceptor processes, including detoxification of xenobiotics and odorants. The age-related decrease in the expression of GSTs, similar to that of cytochrome P-450 (NMa), a phase-I biotransformation enzyme (Getchell et al., *Ann. Otol. Rhinol. Laryngol.* 102:368, 1993) may be associated with the decline in the acuity of odor perception and xenobiotic metabolism in older subjects. Supported by NIH DC-00159 (TVG), DC-01715 (MLG) and P50-AG05144 (ND).

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Cellular Localization of Amyloid Precursor Protein mRNA Isoforms in Rat Olfactory Epithelium.

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Alzheimer's disease is characterized by the presence of neurofibrillary tangles and senile plaques in brain. The major component of these plaques is βA4 or amyloid protein which is derived from a larger precursor protein called amyloid precursor protein (APP). There are three major alternatively spliced forms of APP which are present in various tissues and cell types under normal physiological conditions. APP 695 form is the predominant form in CNS, while APP 751 and 770 forms are abundant in peripheral tissues. The role of APP under normal and physiological conditions and in Alzheimer's disease is not yet understood. Recent studies indicate that the APP 695 form is associated with neuronal differentiation; APP 751 has been implicated in neuronal repair and regeneration and it is upregulated in astroglia following brain injury. Olfactory neurons in the nasal mucosa turn over throughout the life at a slow rate, but accelerated degeneration and replacement of these neurons can be achieved by destroying their synaptic target by olfactory bulbectomy, thereby providing a model for examining the role of APP in neuronal differentiation and repair in vivo. We have previously reported the expression of three major APP isoforms, APP 695, 751 and 770, in the olfactory epithelium. In this study, the ratios of these isoforms were determined by quantitative RT-PCR, and they appear to be different from those in brain. In situ hybridization using digoxigenin labeled riboprobes shows that these isoforms are localized in the neuronal layer of olfactory epithelium. Experiments are being carried out to study the localization of various APP isoforms by using isoform-specific oligonucleotide probes in control olfactory epithelium and during neuronal degeneration and recovery following unilateral bulbectomy.

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The Effect of Human Olfactory Biopsy Upon Olfaction: A Preliminary Report.

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Normal human olfactory function is subject to a wide variety of factors. Although biopsy of human olfactory neuroepithelium has been reported by several researchers, there are no studies which have evaluated the effect of this procedure upon olfactory function. In this retrospective study we sought to determine if tissue removal from the olfactory cleft has an adverse influence upon the sense of smell. Nineteen subjects underwent bilateral olfactory testing and subsequent endoscopic olfactory mucosal biopsy. All of these subjects were retested 6 weeks to one year after olfactory neuroepithelial biopsy. No statistical difference was found between olfactory tests performed before or after biopsy. These data suggest that biopsy of human olfactory neuroepithelium has no discernable adverse effect upon the ability to smell.

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Postnatal changes in the number of olfactory receptor neurons, mitral cells and the possible meaning of the changing convergence ratio.

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In the ferret (*Mustela putorius f. furo*), a carnivorous mammal, olfaction seems to be of major importance. When hunting for prey, ferrets depend heavily on olfactory cues emitted by the prey. However, prey odors have to be learned during a postnatal sensitive phase lasting throughout the third month of postnatal life (Apfelbach R 1986: Behav Proc 12, 363). Light microscopic data reveal that the number of sensory neurons in each olfactory epithelium (septum nasi) increases significantly from about $0.9x10^6$ (neonate) to $25.6x10^6$ (day 60) and decreases thereafter until adult values ($21.6x10^6$) are reached (> 150 days). During the same period the number of mitral cells per olfactory bulb changes from $56.2x10^3$ (neonate) to $73.5x10^3$ (day 60) and thereafter to $79.2x10^3$ (adult value). Thus, the convergence ratio between sensory cells and mitral cells shows age dependent differences and changes from 17:1 to 349:1 and to 273:1. According to this data, the convergence ratio increases by more than 20-fold during the first eight to nine weeks of postnatal life. In addition, there is a remarkable correlation between behavioral and neuronal development: During that developmental stage when the young ferret gets imprinted to prey odors the highest convergence ratio has been found.

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Early Events in Rat Olfactory System Development

QIZHI GONG and MICHAEL T. SHIPLEY *(University of Cincinnati)*

Pioneer olfactory axons emerge from E12 rat olfactory placode and follow a specific pathway to reach the olfactory bulb primordium of the telencephalon in the next 24 hours. At E13-E14, pioneer olfactory axons penetrate into ventricular zone of the telencephalon. The morphology and cellular architecture of the olfactory bulb primordium and neocortex are very similar at E12 and E13. Moreover, there are no known molecular markers to distinguish the olfactory bulb primordium from the rest of the neocortex at these ages. Nevertheless, the pioneer olfactory axons always follow the same route to reach a specific rostral sector of the telencephalon, the olfactory primordium, which develops into the olfactory bulb. Our previous studies demonstrated that the low affinity nerve growth factor receptor (LNGFr) positive olfactory nerve Schwann cells are not present in the olfactory nerve trajectory at E12-E14. Therefore, LNGFr positive olfactory Schwann cells probably do not play a guidance role for the pioneer olfactory axons. Extracellular matrix molecules (ECMs) and cell surface molecules (CSMs) are important in axonal guidance and pathway finding. Thus, we are investigating the temporal and spatial expression of a number of ECMs and CSMs in the early olfactory system to determine if such molecules might be potential guidance for growing olfactory axons. We have examined laminin, fibronectin, chondroitin sulfate conjugated proteoglycan, peanut agglutinin-binding glycoprotein, tenascin, NCAM and L1 at ages E12-E15. Laminin is present sparsely in the olfactory nerve trajectory at E12-E15. Laminin is strongly present in the glial limitans around the telencephalon except the olfactory primordium region. At the entrance of the olfactory nerve to the telencephalon, laminin barrier is perforated. Peanut agglutinin-binding glycoprotein is associated with the olfactory nerve from E12, but this molecule stops abruptly where the olfactory nerve becomes the olfactory layer at E14. Fibronectin and chondroitin sulfate proteoglycan do not appear to be expressed along the olfactory trajectory. At E14, fibronectin is present in the glial limitans outlines the telencephalon, but it is absent where the olfactory nerve enters the telencephalon. Tenascin has a unique pattern of expression: it is expressed on cells in the mantle layer of the telencephalon everywhere except in the olfactory primordium. NCAM and L1 are both expressed on pioneer olfactory axons. To further study the function of these molecules, we are investigating their ability to guide and promote neurite outgrowth in organotypic cultures

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Migration of Luteinizing Hormone-Releasing Hormone (LHRH) and Neural Cell Adhesion Molecule (NCAM)-Immunoreactive Cells From The Epithelium of the Medial Olfactory Pit in Humans. M. SCHWANZEL-FUKUDA and D.W. PFAFF (Rockefeller University, New York, USA); P.M. G. BOULOUX (Royal Free Hospital, London, England); J.-P. HARDELIN and C. PETIT (Pasteur Institute, Paris, France).

Luteinizing hormone-releasing hormone (LHRH) neurons originate in the epithelium of the medial olfactory pit and migrate into the brain along a migration route formed by the NCAM-immunoreactive central fibers of the vomeronasal and terminal nerves (Nature, vol.338,1989; J. Comp. Neurol., vol.321,1992). Originally demonstrated in mice, the migration of LHRH cells has been shown to take place in a variety of mammals, with some species-specific differences in the site of the initial detection of immunoreactive LHRH. In 31 day old human embryos, preceding formation of the olfactory pit and the initial detection of LHRH neurons, NCAM-immunoreactive cells were seen in the epithelium of the olfactory placode, and in no other regions of the surface epithelium of the head. At 35 days, the placode had invaginated to form a simple olfactory pit. NCAM-immunoreactive cells appeared to emerge from the placodal epithelium into the nasal mesenchyme, closely associated with the numerous blood vessels which are present in the anlage of the nose and along the surface of the developing brain at this age . By 39 days, an NCAM-immunoreactive cellular aggregate was seen between the epithelium of the olfactory placode and the rostral tip of the forebrain as predicted from the mouse migration pathway. In 41-42 (but not 38-39) day old human embryos LHRH-immunoreactivity was detected in cells seen in cords in the nasal mesenchyme, just outside of the epithelium of the medial olfactory pit. The LHRH neurons, together with non-LHRH-immunoreactive, NCAM-containing, cells, were seen in and among olfactory placode-derived nerve fiber bundles, presumably the central processes of the terminal and vomeronasal nerves. These LHRH cells appeared to have breached the basal lamina of the forebrain as they followed the NCAM-immunoreactive central roots of the terminal nerve, into the ventral-medial forebrain, caudal to the developing olfactory bulbs.

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Migration of basal cells from the olfactory epithelium after bulbectomy. YUKO SUZUKI AND MASAKO TAKEDA (Higashi Nippon Gakuen University, Ishikari-Tobetsu, Japan.

The basal cell population of the olfactory epithelium, consisting of globose basal cells and basal cells proper (keratin-positive), is the suggested progenitor of olfactory neurons. We studied the migration of basal cells from the olfactory epithelium of postnatal mice by double immunostaining using anti-keratin and anti-bromodeoxyuridine (BrdU) antibodies to label dividing cells, and by conventional electron microscopy. Mice were bulbectomized unilaterally 1-2 days after birth and injected with BrdU 1 hr before sacrifice. Two days after bulbectomy, a few keratin-positive basal cells migrated out of the olfactory epithelium and formed a cord or clusters in the lamina propria. At 5 days, some clusters were present in the intracranial connective tissue. In electron micrographs, the clusters were composed of globose basal cells and basal cells proper. In the olfactory mucosa near the olfactory bulb, many cells including basal cells and immature olfactory cells migrated from the epithelium along Bowman's glands. The migrating cells showed rare BrdU labeling. At 10 days, the clusters were observed between the meninges and the nerve fiber layer of olfactory bulb on the unoperated side. These cells do not express keratin and resemble neuronal and glial cells morphologically. This suggests that the migrating basal cells do not produce olfactory cells but transform into neuronal or glial cells.

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Co-Culture of Olfactory Bulb Cells With Olfactory Epithelial Cells Results in Increased Numbers of OMP-Immunopositive Receptor Neurons. R.J. Grill, Jr. (Univ. of Cincinnati). S.K. PIXLEY (Univ. of Cincinnati, Cincinnati, OH 45267-0521).

The olfactory bulb plays an important role in survival and differentiation of olfactory receptor neurons (ORNs). Recent studies have suggested that contact with the bulb is not an absolute requirement for production of mature ORNs, nor does it stimulate maturation of ORNs. Mature ORNs express the olfactory marker protein, OMP, while immature ORNs express neuron-specific tubulin and GAP43. Instead, contact between ORNs and the olfactory bulb provides trophic support. Contact with the bulb supposedly prevents premature ORN cell death and allows neurons to live through the immature phase, long enough to express the olfactory marker protein, OMP. To test this hypothesis, we have determined the effects of co-culturing dissociated olfactory bulb neurons and dissociated olfactory epithelial cells. First, dissociated, newborn rat, olfactory bulb cells were plated on monolayers of newborn rat cortical astrocytes. Them four days later, we added dissociated adult rat, olfactory epithelial cells. Cells were fixed and immunostained for OMP at 1, 5, 8 and 12 days after plating epithelial cells. After one day, all adult epithelial cell cultures (both single and co-cultures) had higher numbers of OMP-positive neurons than are seen in newborn rat epithelial cultures cultured for the same time. By five days after plating, these numbers had decreased by 95%. At both five and eight days after plating, co-cultures and epithelial cell-only cultures had equivalent numbers of OMP-positive neurons. However, at twelve days after plating, OMP-positive cell numbers in bulb/epithelial co-cultures were four times higher than in epithelial cell-only cultures ($n=3$, $p<0.05$). This was not due to increased cell density in the co-cultures. Cultures with twice the normal number of epithelial cells (equivalent to bulb cultures in total numbers) did not show increased numbers of mature ORNs. In epithelial cell-only cultures, OMP-positive cell numbers did not increase between days 5 and 12. Thus, addition of bulb cells to epithelial cells resulted in a specific increase in the number of mature ORNs. We will now determine if this effect is bulb-specific; does co-culture with other CNS tissues stimulate the same increase?

Supported by NIH grant DC00347.

Uptake and Transport of Cocaine in the Mouse Olfactory System HARRIET BAKER and LINDA FRANZEN. (Cornell Univ. Med. Coll. at Burke Med. Res. Inst.)

As a drug of abuse, cocaine is often self-administered intranasally. Anecdotal evidence has suggested that olfactory acuity is altered by cocaine use. Also, following intravenous administration, cocaine is concentrated in the olfactory epithelium (Brittebo, Tox. App. Pharm. 96:315, 1988). These data, in conjunction with the numerous reports of internalization and transport of substances by olfactory receptor cells following intranasal application, suggested that cocaine might reach the central nervous system via olfactory receptor cell axons. To test this hypothesis, tritium labelled cocaine [up to 25 µCi (250 ng) in 50 µl] was unilaterally instilled intranasally in mice either alone or in combination with unlabelled cocaine (250 ng). Mice were decapitated from 2 to 24 hours after application. Brains and olfactory mucosae were removed from mice and immediately frozen on dry ice. Cryostat sections (25 µm) mounted on glass slides were apposed to (LKB-Ultrofilm-^{3}H) for 10 days to 2 months. Uptake of label into the olfactory mucosa occurred at all concentrations and time points examined. In a few animals, label was primarily unilateral, but in others the turbinates exhibited bilateral labelling. Optimum labelling occurred at about 3 hours. Even in animals where intense label was found in the epithelium, transport from the mucosa into the olfactory bulb was not prominent under any experimental conditions. Fine cellular resolution could not be obtained since diffusion of label occurred in sections post-fixed and dipped in emulsion (NTB-2). These data suggest that cocaine is internalized by the olfactory mucosa but that, at best, limited transport occurs to the brain. Thus, access of cocaine to the brain is not significant by this route. Supported by DC01710.

Tobacco Smoke-Induced Alterations of Rhodanese and Carboxylesterase in the Olfactory Mucosa of F344 Rats.
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Cigarette smoke contains a complex mixture of toxicants known to alter the quantity and functional activity levels of enzymes in a variety of tissues. Because the nose, the portal of entry for inhaled toxicants, has a high capacity for xenobiotic metabolism, we examined the effect of cigarette smoke inhalation on activity and localization of the xenobiotic-metabolizing enzymes rhodanese (RH) and carboxylesterase (CE) in the olfactory mucosa, the site of greatest metabolic capacity of RH and CE. These enzymes metabolize cyanide (RH) and esters (CE) known to be present in cigarette smoke. Thirty, 6-wk old CDF®(F344)/CrlBR rats were exposed to filtered air (controls) or mainstream 1R3 research cigarette smoke at concentrations of 100 (low smoke, LS) or 250 (high smoke, HS) mg total particulate matter/m^3 for 6 h/day, 5 days/wk, for 32 wk. Ethmoturbinate sections (lined by olfactory mucosa) were stained for morphologic examination or reacted with polycolonal antibodies to RH or CE, or with normal sera (negative control slides). Activity levels of RH and CE were determined biochemically in mitochondrial and S-9 preparations of ethmoturbinates from control and exposed rats. Proteinaceous globules occured in sustentacular cells following both LS and HS exposures. These globules were not RH immunoreactive, but were intensely CE immunoreactive. LS rats showed a decrease in RH immunoreactivity in sustentacular cells. RH immunoreactivity was increased in those sustentacular cells that did not contain globules in HS rats. Changes in RH Vmax corresponded to these differences in immunoreactivity; Km changed inversely. In LS rats, induction of CE in globules correlated with an increase in CE Vmax. In HS rats, the increase of CE in globules was balanced by the loss of CE in Bowman's glands in areas of mucosal attenuation that occurred with HS exposure, resulting in no change in CE Vmax. Km increased slightly in LS rats and was unchanged in HS rats. In conclusion, inhalation of tobacco smoke induces complex lesions that result in either enzyme induction or reduction from specific cells found in the olfactory mucosa of rats. Despite these alterations, the metabolic rates of RH or CE at low substrate concentrations (Vmax/Km) were not changed in smoke-exposed rats compared to controls.

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Glycogen, a Polysaccharide with a Taste to Hamsters? **BRUCE I. MACKINNON, MARION E. FRANK (University of Connecticut Health Center, Farmington, CT, USA) and BRADLEY G. REHNBERG (York College, York, PA, USA).**

The idea that polysaccharides have a taste was explored with behavioral and neurophysiological studies on hamsters (Mesocricetus auratus). A commercial preparation of oyster type II glycogen, a 270KD to 3,500KD glucose-based polysaccharide that is highly preferred, elicits a sizable chorda tympani response. However, 8.8KD to 2,000KD dextrans, which are also glucose-based polysaccharides, produce no chorda tympani response at 3.2%. Dialysis at 7KD removes 15% of 3.2% glycogen and reduces the chorda tympani peak response by two-thirds, indicating that glycogen itself elicits little of the chorda tympani response. Since a 3.2% glycogen solution also contains 3.7mM Na^+, 2.3mM K^+, and 1.3mM Ca^{++}, a large part of the chorda tympani response may be due to contamination of the glycogen material. The chorda tympani response to 10mM NaCl is reduced by 50% with 10uM amiloride pretreatment, but the response to 3.2% glycogen is reduced by only 21%, supporting the idea that sodium is not the only effective stimulus. Deionization of 10% glycogen with a mixed-bed ion exchanger reduces conductivity of the solution by 85% and the chorda tympani response by 26%. The data suggest chorda tympani stimulation by substances other than ions. Presently, single fiber responses to glycogen are being analyzed to determine the role of fiber type on the whole nerve response, which will indicate the importance of smaller saccharides. Although the chorda tympani whole-nerve response to glycogen is reduced by dialysis, hamsters still prefer dialyzed glycogen to water.
[Supported by NIH grant DC00058]

Ionic Contaminants and Polycose Taste Responses. B.K. FORMAKER, C.J. HARP, B.I. MACKINNON, T.P. HETTINGER & M.E. FRANK (The University of Connecticut Health Center)

Electrophysiological taste recordings from the rat nucleus of the solitary tract (NST) indicate that Polycose, a starch derived polysaccharide, is more likely to stimulate salt and acid best units than sugar best units (Giza, et. al., 1991). However, Polycose contains sodium, calcium, potassium and chloride in concentrations that can be effective as gustatory stimuli. The current study examined the role of these ionic "contaminants" on electropysiological and behavioral taste responses in the golden Syrian hamster (Mesocricetis auratus). Multi-fiber chorda tympani (CT) responses to 10% deionized Polycose were obtained from 4 adult male hamsters. Behavioral data was obtained using a conditioned taste aversion procedure. Animals were conditioned to avoid either 10% Polycose or 10% deionized Polycose. Control animals were conditioned against water. Generalization stimuli consisted of both Polycose solutions, 0.03M NaCl, 0.3M KCl and 0.1M sucrose. Ionic components were removed from Polycose by passing a 10% Polycose solution through a mixed-bed ion exchanger. Removing the ions from Polycose reduced the mean peak CT response 80%. Thus, the ionic components of Polycose make significant contributions to CT responses and presumably to NST responses also. Behaviorally, aversions to deionized Polycose generalized to Polycose and vice versa, but not to NaCl or KCl. Animals conditioned to avoid deionized Polycose significantly suppressed intake of Polycose 67% and deionized Polycose 66%. Sucrose intake was significantly suppressed 45%. Thus, Polycose may share some taste quality with sucrose in the hamster. Currently, single fiber analyses are being conducted to delineate the tuning characteristics of CT fibers responsive to deionized Polycose.
[Supported by NIH Grant DC00058]

IBMX and Hodulcin-Induced Suppression of Receptor Cell Responses to Sucrose in *Phormia Regina*.

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Hodulcin (HDE), from the leaves of *Hovenia dulcis*, suppresses behavioral and receptor cell potential responses to sucrose in flies (Kennedy et al., 1988, Kolodny and Kennedy, 1988). It has been suggested that this effect is achieved by inhibition of a later step in a multistep process (Frank, et. al, 1992), such as a cyclic adenosine or guanosine 3'5' monophosphate cascade (Kennedy and Kolodny, 1994). Moreover, some sweet tasting compounds are known to activate adenylate cyclase by a mechanism that requires the presence of guanine nucleotides (Striem et al., 1986, 1989). In an attempt to determine if HDE suppression of receptor cell action potential responses to sweet tastants is mediated by action on a molecule in such a cyclic nucleotide cascade, single fly taste sensilla were treated for 2 min with Tris, pH 7.0, 0.001M of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), 0.05% w/v HDE, or a mixture of the above solutions (drugs were dissolved in 25mM Tris, pH 7.0. After each drug treatment, there were significant suppressions of receptor cell responses to 50mM sucrose during the 10 min test period (Kramer ANOVAs, $p<0.01$). There was no significant difference between groups in median ratios of responses prior to treatment. However, at 1, 3, 4, 5, 6 and 7 min post treatment, there were significant differences between the groups. Initially, all three drug treatment groups exhibited suppressed receptor cell responses to sucrose, and the inhibition produced by the HDE/IBMX mixture appeared to be the sum of the effects of HDE and IBMX. HDE treated sensilla recovered after 3 min, but IBMX-treated sensilla remained suppressed for 8 min ($p<0.05$, Kruskal-Wallis Tests). Median ratios of sensilla treated with the mixture continued to be additive until 8 min post treatment. Studies with other agonists and antagonists of the cyclic nucleotide cascades are in progress.

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Contribution of Amiloride-Sensitive Pathways to Acid Transduction in Rats.

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Amiloride-sensitive (AS) Na^+ channels have been implicated recently in the transduction of acidic stimuli in hamsters using giga-seal whole cell recording, extracellular loose patch recording from taste buds *in situ*, and behavioral measures. Because the Na^+ content in the saliva of rats is significantly higher than that of hamsters (Rehnberg et al., *Chem. Senses* 17:179, 1992) and Na^+ and protons interact at the AS Na^+ channel (Gilbertson et al., *J. Gen. Physiol.* 100:803,1992), differences might be expected between rats and hamsters with respect to acid responses. In contrast to hamsters, few action potentials are generated in response to acid stimulation (citric acid, pH 2.6) during *in situ* recording from rat fungiform taste buds. Four of 23 taste buds generated action potentials during acid stimulation and these were blocked by amiloride (30μM), similar to the effect seen in hamster. More typically, acid stimuli caused subthreshold increases in electrical activity (10/23 taste buds). These responses were never blocked by addition of amiloride to the stimulating solution. The remaining taste buds did not respond to acid stimulation although they responded with generation of action potentials to NaCl (100-200 mM). To determine if amiloride could ameliorate the aversiveness of acids behaviorally, 4 day two-bottle preference tests were conducted on male rats in groups of 6-12. Acid solutions were avoided relative to water in a dose-dependent manner with decreasing pH from a threshold between pH 3.5-4.0. Addition of amiloride (30,300 μM) had no significant effect on rejection of the acid solutions at any pH. Taken together, these results suggest AS pathways may not contribute significantly to acid transduction in rats and point to the involvement of additional transduction mechanisms for acids.

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Quinine Suppresses Facial Taste Responses to Amino Acids in the Channel Catfish.

K. OGAWA AND J. CAPRIO (Louisiana State University)

This study evaluated the effect of Quinine-HCl (QHCl) on the activity of 26 amino acid-responsive, single facial taste fibers innervating the maxillary barbel of the channel catfish, *Ictalurus punctatus*. All fibers tested were most responsive to either 10^{-4}M L-alanine (Ala-best; n=16; 61%), 10^{-4}M L-arginine (Arg-best; n=9; 35%) or 10^{-3}M L-proline (Pro-best; n=1; 4%). Dose-response analysis of twenty single facial taste fibers indicated that six fibers (30%) were excited (group I) and 14 fibers (70%) were suppressed (group II) by QHCl. All group I fibers were Ala-best, whereas group II fibers were composed of six Ala-best and 8 Arg-best fibers. Binary mixtures of 1 mM QHCl and L-alanine (10^{-8}M to 10^{-3}), L-arginine (10^{-7}M to 10^{-2}M), and L-proline (10^{-3}M to 10^{-1}M), respectively, were tested. QHCl inhibited the responses to L-arginine in group I fibers by 33%, whereas QHCl suppressed taste responses of group II fibers to L-alanine by 74%, to L-arginine by 53% and to L-proline by 57%. This suppressing effect of QHCl when used as a binary stimulus was quickly reversible in subsequent testing of stimuli in the absence of QHCl. QHCl also suppressed the responses to stimuli other than amino acids, such as $\leq 10^{-1}$M caffeine and $\leq 10^{-1}$M denatonium benzoate (DB); however, neither caffeine nor DB when used in a binary mixture with either L-alanine, L-arginine or L-proline had any depressing effect on amino acid taste responses. QHCl also suppressed both taste and tactile responses of bimodal facial taste fibers, but had no effect on tactile-only (possibly facial and/or trigeminal) fibers. The mechanism for the suppression effect of QHCl on taste fibers is unknown, but it is possibly related to quinine's lipophilic property as reflected in the nonspecificity of its described actions: (a) suppression of responses to amino acids that bind to independent taste receptor sites (Caprio et al., *TINS* 16:192-197, 1993), (b) suppression of taste activity to other bitter stimuli, (c) suppression of both taste and tactile responses of bimodal facial fibers and (d) suppression of spontaneous activity of facial taste fibers.

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Responses of Single Glossopharyngeal Taste Fibers in the Channel Catfish, *Ictalurus punctatus* to Amino Acids.

K. OGAWA and J. CAPRIO (Louisiana State University)

Knowledge concerning the processing of amino acid taste information in fishes is derived almost exclusively from facial (VII) nerve recordings. Only two investigations reported the activities of glossopharyngeal (IX) and vagal (X) taste neurons to amino acids and contrasted those with VII taste activity in the same species (Sutterlin and Sutterlin, 1970; Kanwal and Caprio, 1983). The multiunit study by Kanwal and Caprio (1983) reported that taste buds innervated by any of the three cranial taste nerves in the channel catfish were most responsive to the amino acids, L-alanine, L-arginine and L-proline, suggesting that these stimuli provide important cues for both appetitive (VII) and consummatory (IX) patterns of feeding behavior. The present study describes the response characteristics of 64 single IX taste fibers in the channel catfish to amino acids and contrasts the results with those of 133 single VII taste fibers in the same species (Kohbara et al., 1992; Ogawa and Caprio, unpublished). Hierarchical cluster analysis of the standardized number of action potentials elicited to 1mM stimuli during a three second response time identified three major groups of IX taste fibers, i.e. neurons highly responsive to L-alanine (Ala cluster; n=17), to L-arginine (Arg cluster; n=23), and to L-proline (Pro cluster; n=24). These results indicate a major percentage increase [up from 3% (VII) to 38% (IX)] in IX neurons comprising the Pro cluster at the expense of IX neurons within the Ala cluster [down from 53% (VII) to 26% (IX)]. The percentage of fibers within the Arg cluster of both VII (40%) and IX (36%) fibers were similar. Dose-response characteristics for L-alanine, L-arginine and L-proline of representative IX taste neurons comprising the Ala and Arg clusters were highly similar to those of representative VII taste neurons (Kohbara et al., 1992). Representative neurons within the Pro cluster were characterized by steep dose-response functions for L-proline and L-alanine occurring at concentrations > 0.1mM for L-proline and > 1.0mM for L-alanine and L-arginine; at 100mM, the responses to proline and alanine were similar and approx. 45% greater than those to L-arginine.

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Bitter and Sweet Taste in Chimpanzee and Rhesus Monkey
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Yuzo Ninomiya, (Asahi University, Gifu Prefecture, Japan).

The relationship between the bitter and sweet taste qualities is intriguing for several reasons. For example, in most cases a minor change of the structure of a sweet compound not only changes its taste quality from sweet to bitter, but also changes its hedonic quality from attractive to avoided. Relating chemical changes of tastants with the effects on their taste can be a powerful tool in receptor modelling, either as one uses an animal model (Hellekant and Walters, 1993) or humans.

However, when using animals it has to be kept in mind that differences in the sense of taste of an animal and that of a human may skew the applicability of the animal results to humans. We have documented this repeatedly for the sweet taste in primates. Most recently in two Madagascian monkeys (Hellekant et al., 1993a, b). Fortunately, the closer related the animal species used is to humans, the more applicable the results are. Consequently, as one uses animal models more and more closely related to humans, the data should be more and more applicable on the human sense of taste.

We will present electrophysiological data from rhesus monkey and chimpanzee and discuss bitter and sweet taste in the light of taste fiber data.

Hellekant, G., Walters, D. E., On the sweetness of five high-potency sweeteners in rats. In "Sweet Taste chemoreception". eds. Mathlouthi, M., Kanters, J.A., Birch, G. G., M., Parthenon Publishing Ltd. 1993. 371-384. Hellekant, G., C. M. Hladik, V. Dennys, B. Simmen, T. W. Roberts, and D. Glaser., On the relationship between sweet taste and seasonal body weight changes in a primate (*Microcebus murinus*). Chem. Senses. 1993. 18. 27-33. Hellekant, G., C. M. Hladik, V. Dennys, B. Simmen, T. W. Roberts, D. Glaser, G. DuBois, and D. E. Walters, On the sense of taste in two malagasy primates *(Microcebus murinus and Elemur mongoz)*. Chem. Senses. 1993. 18. 307-320.

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Amiloride reduces the aversiveness of acids in preference tests.
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Recent work using both *in situ* taste bud recording (Gilbertson et al., *J. Gen. Physiol.* 100:803, 1992) and patch clamp recording from isolated hamster taste receptor cells (Gilbertson, et al., *Neuron*, 10:931, 1993) has shown that amiloride-sensitive (AS) Na channels are permeable to protons. These results suggested that AS pathways contribute to the detection of acids in hamster. Using two-bottle preference testing, experiments were conducted to determine the relative contribution of AS Na channels to the transduction of acid (sour) stimuli. Hamsters were given a choice between water and varying concentrations of citric acid in preference tests lasting 4 days. At pH 2.0 and below citric acid was avoided by all animals; the concentration of citric acid which was half-maximally aversive was at pH 2.4. To test for the involvement of AS pathways in the detection of citric acid, preference tests were conducted in the presence of amiloride. Amiloride (30, 300 μM) could significantly reduce the aversiveness of both citric acid (pH 2.4) and HCl in a dose-dependent manner, similar to its effects on reducing the aversion to NaCl solutions. Amiloride, however, had no effect upon the preference for saccharin solutions and was itself neither preferred nor avoided relative to water. This effect is consistent with the physiological findings which suggested that AS Na channels contribute to the transduction of acidic (sour) stimuli. At high concentrations (300 μM), however, amiloride could not completely ameliorate the aversiveness of citric acid solutions. This finding suggests that additional mechanisms for the transduction of acids are present and may also contribute to the perception of acids in hamsters.

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Catfish Taste Cell Calcium Changes in Response to Arginine.
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Taste receptor cells are modified epithelial cells that respond to taste stimuli by modulating release of neurotrasmitter at synapses with afferent fibers. Intracellular calcium is presumed to be involved in neurotransmitter release from taste cells. In catfish, some amino acids are potent taste stimuli, and L-Arginine (L-Arg) activates a nonspecific cation channel that is permeable to calcium. We measured the intracellular concentration of calcium in isolated catfish taste cells using the calcium indicator Fura-2. Taste cells were isolated from maxillary barbels by enzymatic treatment with collagenase and hyaluronidase followed by incubation in divalent cation-free fish Ringer's solution. Cells, attached to a Convalin A or poly-Lysine covered glass slide, were constantly perfused with a fish Ringer's solution. Stimuli were delivered by switching the perfusing solution. L-Arg induced changes in intracellular calcium in seven out of 60 cells. Five cells showed a decrease and the other two exhibited increases. The L-Arg-stimulated increase in calcium was antagonized by eqimolar D-Arginine (D-Arg), consistent with the inhibition of L-Arg stimulated channels by D-Arg. D-Arg has also been shown to be a stimulus. D-Arginine induced responses in 8 out of 25 cells. Seven cells exhibited an increase in intracellular calcium, while one cell showed a decrease. The response to D-Arg was neither blocked nor enhanced by the introduction of L-Arg. About half of the cells tested showed a rapid transient increase in intracellular calcium when calcium was removed from the perfusing Ringer's solution. Similar transients were observed in a few cells when a stimulus was removed. These experiments are consistent with the presence of multiple pathways for arginine taste transduction in catfish. The calcium responses to L and D arginine appear to be mediated by independent receptors.

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Immunoaffinity Isolation of a Putative L-Arginine Taste Receptor from the Channel Catfish.
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The channel catfish, *Ictalurus puntatus*, possesses at least two distinct taste receptor systems for amino acids. One recognizes L-alanine and other neutral amino acids, the other sensitive to L-arginine. This latter receptor has been well characterized as a stimulus-gated ion channel type receptor. Previous studies demonstrated that PHA-E lectin selectively inhibited binding of L-arginine, but not L-alanine, to taste receptors of the channel catfish (Kalinoski, et al. Chem. Senses 17:381-391, 1992). Using PHA-E chromatography we isolated 4 proteins from taste epithelial membranes and raised antibodies against these (Kalinoski et al. ISOT XI, abstr, 1993). Antibodies to one of them, a band of M, 33,000 (anti-R2), specifically localized to the apical surface of catfish taste buds and also suppressed L-arginine-activated conductances in taste membrane fractions reconstituted into lipid bilayers. However, the anti-R2 antibody also recognized PHA-E lectin that had copurified with the catfish epithelial proteins. We have subsequently isolated a taste membrane specific immunoglobulin fraction (LA-R2) from anti-R2 by lectin absorption. Like native anti-R2, the lectin-absorbed antibody localized to the apical surface of the taste bud and suppressed L-arginine-activated conductances. Following immunoaffinity chromatography of taste epithelial membranes fractions using LA-R2, SDS-PAGE demonstrated the presence of a single protein band of M, 33,000. When this fraction was reconstituted into lipid vesicles by extensive dialysis and then into lipid bilayers, L-arginine-activated conductances were observed. These results suggest that the M, 33,000 protein isolated by LA-R2 immunoaffinity chromatography is a component of the L-arginine receptor/channel.

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Active Site Mapping and Ligand Binding Studies of a Insect Odorant Binding Protein

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Mapping of the pheromone binding site in the recombinant pheromone binding protein (PBP) of *Antheraea polyphemus* was accomplished using photoaffinity labeling by analogs of two pheromone components. With [^{3}H]*6E,11Z*-hexadecadienyl diazoacetate, a photoactivable analog of the naturally-occurring acetate pheromone, labeling occurs primarily within the hydrophilic domain Asp^{39}-Arg^{46} adjacent to the highly-conserved hydrophobic region Leu^{47}-Lys^{58} which was suggested as a recognition site for the lipophilic hydrocarbon backbone of pheromones. Edman degradation located the covalently attached ligand at Thr^{44} residue. For the photoaffinity analog [^{3}H]*4E,9Z*-tetradecadienyl diazoacetate, the hydrophobic region Asp^{21}-Lys^{38} adjacent to the binding domain Asp^{39}-Lys^{58} contained a second modification site. The fourteen-carbon odorant molecule was able to occupy two positions within the recognition site, while a single binding position was available to the sixteen-carbon pheromone.
A new approach was designed to solve the problem of quantification of pheromone binding in aqueous solution by coating the surface of an assay tube with an n-alkyl alcohol. The binding affinity of the recombinant PBP for the radiolabeled pheromone [^{3}H]*6E,11Z*-16:Ac was determined by Scatchard analysis (Kd=0.64 μM). Competition assay with selected ligands of various chain length, saturation and functional group showed the binding selectivity of PBP. This technique has general applications for odorant binding proteins and their putative ligands.

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Olfactory Processing In The Honey Bee, *Apis mellifera*: Sensory interactions between odorants in binary mixtures

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A primary challenge facing the olfactory system is to filter-out irrelevant background odorants in the detection of odorants relevant to survival. The mechanisms involved in accomplishing this task are of interest because of the surprising structural similarity between olfactory processing centers in a wide array of animals. Recently, Smith & Cobey (in press) demonstrated in the honey bee that perceptual processing of an odorant is dependent on several conditions regarding the odor background in which it was trained. This "overshadowing" effect of odorant background can result from any of several peripheral and central neural mechanisms, beginning with sensory interactions among odorants in a mixture. To test whether sensory interactions could account for overshadowing effects, we used electroantennogram (EAG) recordings to measure the potential change across the antenna that results from odorant stimulation. We predicted that adaptation of the EAG response to an odorant by repeated stimulation in rapid succession would generalize to other odorants for which there is strong cross-fiber interactions with the adapted odorant and that these patterns would correlate to behavioral data on overshadowing. When bees were tested with floral odorants, the EAG responses were a function of concentration for hexanal, 1-hexanol and octanal but not for 2-hexanone. Sensitivity to 2-hexanone at very low concentrations may be due to its structural similarity to 2-heptanone, a component of a honey bee alarm pheromone. A significant cross-adaptation was observed for odorants that show behavioral overshadowing effects and was dependent on concentration of the adapting odorant for all four odorants – hexanol, 1-hexanol, 2-hexanone and octanal. That is, cross-adaptation was limited to higher concentrations of the adapting odorant. These results support the interpretation that some overshadowing effects can be explained by cross-fiber coding of odorants. Studies presently underway will investigate whether cross-adaptation is observed in mixtures of floral odorants and pheromones, for which no overshadowing is observed in behavioral studies. These studies highlight the importance of odorant concentration in studies of mixture processing.

Smith, B.H., Cobey, S. in press. Olfactory processing in the honey bee, *Apis mellifera*. II. Blocking between odorants in binary mixtures. J. exp. Biol.

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Multiplicity of Salmonid Olfactory Receptors for Bile Acids as Evidenced by Cross-adaption and Ligand Binding Assay

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Bile and bile acids are potent olfactory stimuli in salmonids, which is implicated in chemical signals in fish behaviors. Our electrophysiological studies have shown that olfactory sensitivities to bile acids are highly specific, receptor-mediated processes in salmonids. All bile acids tested fall into three groups (free, conjugated and sulfated bile acids) based on their distinct concentration-response relationships, suggesting the existence of three separate receptor mechanisms. To investigate bile acid receptors, we conducted electroolfactogram (EOG) cross-adaption experiments and ligand binding assay in lake char, *Salvelinus namaycush*. Reciprocal cross-adaption experiments on bile acids, amino acids and prostaglandins demonstrated that receptors for bile acids are independent of those for others. Olfactory responses to bile acids were inhibited by other bile acids within the group but not by other groups', suggesting existence of three receptors specific for free, conjugated and sulfated bile acids. Thus, observed olfactory responses are the result of combined activities of an individual bile acid that could interact with these three relatively independent receptors. Ligand receptor binding assay was carried out on slide-mounted olfactory rosette sections by using [^{3}H]taurocholic acid. Binding saturability, reversibility and affinity were reasonably met receptor criteria. Specific binding was markedly reduced in deciliated tissues and eliminated after olfactory nerve section. Kinetic analysis of saturation and competition curves revealed three receptor binding components. The accordance between ligand binding data and EOG cross-adaption results supports our three bile acid receptor mechanisms hypothesis.

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Inhibition of Odorant-Receptor Binding Predicts Binary Mixture Interactions for Glutamate Receptors But Not Taurine or 5'AMP Receptors in the Olfactory Organ of the Spiny Lobster.

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Mixture interactions, in which responses to mixtures are significantly greater or less than expected based on measured responses to the mixtures' components, occur in many species including the spiny lobster Panulirus argus. Previous electrophysiological experiments have shown that olfactory receptor neurons (ORNs) of the spiny lobster express mixture interactions, primarily mixture suppression, and with specificity (i.e., only specific combinations of odorants affect specific types of ORNs). We examined the possibility that a suppressive odorant can mediate its mixture effects on ORNs by inhibiting binding of an excitatory odorant to its receptors. We examined this for three receptor systems -- L-glutamate, taurine, and 5'AMP -- and for binary mixtures containing 5'AMP, ammonium, betaine, L-cysteine, L-glutamate, DL-succinate, and taurine. This was accomplished by comparing the ability of each of the above 7 odorants to inhibit binding of tritiated glutamate, taurine, or 5'AMP (measured using a biochemical radiolabeled odorant-receptor binding assay), against the ability of these same 7 odorants to either suppress or excite glutamate-best, taurine-best, or 5'AMP-best ORNs (measured using single-unit electrophysiological techniques). We found a significant correlation for glutamate receptors but not for either taurine or 5'AMP receptors. This analysis suggests that mixture suppression of responses of ORNs to glutamate by other odorants may be a result of the other odorants inhibiting binding of glutamate to its receptors, but mixture suppression of responses of ORNs to taurine or 5'AMP is likely due to other mechanisms.

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Synergism of Insect Sex Pheromone Specialist Olfactory Receptor Neurons by Synthetic Analogues and Sex Pheromone Components.

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Two synthetic sex pheromone analogues and two sex pheromone components each synergize the response of the HS(a) sex pheromone olfactory specialist receptor neuron of the male cabbage looper, Trichoplusia ni (Hübner) to (Z)-7-dodecenyl acetate, the most behaviorally active component of the sex pheromone. The same compounds also synergized the (Z)-11-hexadecenal specialist receptor neuron of the corn earworm moth, Helicoverpa (=Heliothis) zea (Boddie). Assays of upwind anemotactic flight and mating behavior of T. ni with the same mixtures that were used in the electrophysiological studies deployed at the same airborne stimulus concentrations showed that the synergism of the receptor neuron is reflected by synergized amounts of behavioral responses. Synergism was achieved at lower airborne stimulus concentrations with the pheromone analogues than with the two sex pheromone components. The airborne stimulus concentration of the pheromone components that was necessary to effect synergism was greater than that emitted by females. As a consequence it is not readily apparent whether or not synergism plays a part in pheromone mixture discrimination. Most of the effective synergist molecules have both a hydrocarbon end and a polar end. Several hydrocarbons were not effective synergists.

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Responses of a Population of Olfactory Receptor Cells to Binary Mixtures in the Spiny Lobster. PETER C. DANIEL (Hofstra University), AND CHARLES DERBY (Georgia State University)

We have shown that mixture interactions towards binary mixtures of odorants may be common in olfactory receptor cells in spiny lobsters (Derby et al, 1991, J. Neurophysiol. 66:112-130,131-139). These interactions can result in suppression of population response intensity as well as modifications in the across neuron pattern of response (ANP) called **pattern mixture interactions (PMIs)**. However, this interpretation is equivocal for two reasons: the study design was limited to one subset of the recorded population of receptor cells, namely those cells whose maximum response was for one of two stimuli comprising the binary mixture; and test concentrations of components varied across cells in a population. In the present study, we recorded extracellularly responses of 50 **randomly selected** individual olfactory receptor cells to **equimolar concentrations** (0.5 mM) of seven compounds (AMP, betaine, cysteine, glutamate, NH_4, succinate, taurine), binary combinations of these stimuli at the same concentrations, and 0.5 mM concentrations of two complex mixtures: artificial crab and oyster. Predicted responses to binary mixtures were based on a noncompetitive (multiple) receptor model and a competitive (single) receptor model. As in other studies, recorded cells were narrowly-tuned toward one of the single compounds. The receptor population showed significant suppression toward 4 of 21 mixtures and 10 of 21 mixtures by multiple receptor and single receptor models, respectively. Magnitude of suppression is independent of cell type (i.e., cells sharing the same spectral characteristics). Thus all cell types may contribute to mixture interactions. A multidimensional scaling (MDS) procedure was performed on observed and predicted responses of all cells to all stimuli in order to analyze PMIs. In contrast to the previous study, observed and predicted ANPs for responses to most binary mixtures were similar. We are determining whether differences in cell types used in the MDS analysis (i.e., best cells only versus all cell types) as well as other factors may account for differences in results of the two studies.

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Computer Modeling of Odor Ligand-Odor Receptor Interactions.

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Sequence comparison has shown that olfactory receptors belong to the superfamily of seven transmembrane domain (7TD), G protein-coupled receptors (GPCRs). By analogy with these systems, it is believed that odor reception involves interactions between odor ligands and residues at specific sequence positions, but thus far there has been no test of this hypothesis with molecular modeling techniques.

Computer-generated three-dimensional models of odor receptors were constructed with a bacteriorhodopsin template using conventional software. Although agonist odor ligand(s) for a given receptor sequence have not been determined, we hypothesize a broad-range affinity of odor receptors, suggesting a strategy of comparing the relative binding strengths of a panel of carefully selected odor ligands to potential ligand binding residues. In this report we focus our analysis on the rat I9 receptor (Buck & Axel, 1991), a representative mammalian odor receptor, in complex with benzaldehyde, the odor ligand for which the receptor showed the greatest affinity, and compare it with binding of the beta-adrenergic receptor (bAR) as the best studied GPCR for its agonist epinephrine .

A group of ligand binding residues of the I9 receptor formed a binding pocket approximately 12 Å from the extracellular surface of the receptor. Docking of the other ligands in the panel also occurred in this general region, supporting the hypothesis that odor receptors form a binding pocket similar to that formed by the other members of the GPCR superfamily. The most important residues within the pocket for binding of benzaldehyde were Tyr 278, which formed a hydrogen bond with the carboxyl group of benzoic acid; Phe 104, which formed an aryl-aryl interaction with the phenyl ring; and Leu 245, which formed van der Waals interactions. Phe 104 corresponds to the aspartate conserved in helix III of aminergic and cholinergic receptors (bAR 113), which acts as a counterion the cationic amine of the ligand. Leu 245 corresponds to a conserved aromatic residue in helix VI of other GPCRs that is implicated in hydrophobic shielding of charged groups (bAR 289).

The results thus provide evidence for several potential ligand binding residues in Helices III through VII of the odor receptor that form a binding pocket. This approach should provide insight into the molecular mechanisms of odor recognition and discrimination, and provide a basis for future experimental analysis.

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Study of Salamander and Rat Olfactory Receptor Physiology Using Voltage Sensitive Dyes. ROBERT C. GESTELAND, PEGGY FARMER and MAUREEN FITZGERALD (University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521).

Fluorescence intensity changes in olfactory receptor neurons stained with the voltage-sensitive dye RH-795 (Molecular Probes, Inc.) are used to measure responses to odors. Tissue viability and magnitudes of potential changes are determined by measuring responses to changes in extracellular $[K^+]$. A laser scanning confocal microscope is used to image the medial septal rat and dorsal and ventral salamander olfactory epithelia. Magnification is adjusted so that each neuron dendrite cross section encloses 460 pixels. Changes of membrane voltage 1 mV and greater are detected. Odors are presented as 1 sec duration vapors to the epithelial surface. Images are acquired every sec for 20 sec for each stimulus presentation. Between presentations the epithelium is in an oxygenated Ringer bath. Vapors presented at dilutions of 1:30 and higher evoke mostly depolarizing responses which are repeatable. Response magnitude increases with stimulus concentration. At higher concentrations hyperpolarization or no response result from presentations after the initial depolarizing response. Different cells differ in response time course to the same stimulus. Some cells return to the resting state within a few sec. Others remain depolarized for more than 20 sec. Analysis of 364 salamander neurons presented with 4 stimulus substances showed that 4% responded to all 4 odors, 16% responded to 3 odors, 42% responded to 2 odors, 24% responded to 1 odor and 14% responded to none.

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Transgenic Analysis of the OMP Promoter Illustrates Differential lacZ Expression in Discrete Subsets of Olfactory Neurons. ERIC WALTERS, GLAUCO TAROZZO, ALICIA PHILLIPS, MARY GRILLO, and FRANK MARGOLIS (Roche Institute of Molecular Biology, Nutley, NJ).

Recent reports demonstrate that the primary olfactory sensory epithelium is comprised of distinct subsets of olfactory neurons. This is evident from the distribution of physiologic responses, of antigen/carbohydrate binding epitopes, and of putative odorant receptors. While using transgenic mice to define 5' regulatory elements of the OMP gene, we have identified lines of mice that target transgene expression to distinct subsets of olfactory receptor neurons. Lines carrying approximately 0.8 kb of upstream 5' promoter fused to the E. coli β-galactosidase (lacZ) gene expressed lacZ primarily in dorsal anatomical regions of the olfactory mucosa when coronal sections were stained by X-gal histochemistry. Whole mount analysis of this tissue revealed significant expression in nasal endoturbinates II and IV. Most strikingly, in one line carrying 0.3 kb of 5' OMP promoter, there was significant lacZ expression in olfactory neurons of endoturbinate II'; this corresponded to robust staining of a subset of glomeruli in the olfactory bulb. The patterns of lacZ expression in these transgenic lines appear to resemble those of putative odorant receptors, in that they are also expressed in a bilateral, symmetric fashion. Endogenous OMP expression in olfactory neurons was normal in all transgenic lines and co-expression of OMP and β-galactosidase was consistently observed. The zonal, topographic expression of lacZ in these lines suggests that these transgenes are integrated into genetic loci involved in 1) regulating developmental organization of the olfactory neuroepithelium and/or 2) putative odorant receptor expression.

Odorant Transport and Uptake in a Bullfrog Nasal Cavity Model KEE H. PYON[1], PETER W. SCHERER[1], MAXWELL M. MOZELL[2,3] & GEORGE PRETI[3] ([1]Dept. of Bioengineering, Univ. of PA, Phila., PA; [2]SUNY Health Sci. Ctr. at Syracuse; & [3]Monell Chem. Senses Ctr. & Dept. of Dermatology, Univ. of PA, Phila., PA).

A scaled-up (52-fold) anatomically correct model of a bullfrog (*Rana catesbeiana*) left principle nasal cavity has been constructed in order to study the pattern of odorant distribution. Experiments done by Mozell et al. (Chem. Sens. 16, 631-649, 1991) have shown that the flow rate of odorant into the bullfrog nasal cavity and the solubility of the odorant with the olfactory mucosa contribute significantly to the odorant patterning activity as measured by recordings from the olfactory nerve. Three odorants (carvone, geraniol, and d-limonene) of varying solubilities in real bullfrog olfactory mucosa were studied at two flow rates. High and medium sniff flow rates were simulated in the model by kinematically matching flow parameters in the real and model nasal cavity. Guaran gel was used as the mucus simulant applied on the walls. Odorants were introduced through the external naris of the model, collected from the free air stream in various regions throughout the model, and then analyzed by gas chromatography/mass spectrometry. Results showed that for carvone (highly soluble in bullfrog olfactory mucosa), at the front part of the model, the concentration of the airphase odorant was higher at the high flow rate than at the medium flow rate. Geraniol (medium solubility) showed opposite results in that there was higher airphase concentration at the medium flow rate. Finally for d-limonene (less soluble than carvone and geraniol), high concentration was detected at most of the regions for both flow rates. The high airphase concentration may reflect the volatility of d-limonene as well as less solubility in the mucus simulant. These odorant collections through our large scale model show that there is a relationship between the effect of flow rate and the sorption strength of the odorant, going from a negative effect for the less sorbed odorant to a positive effect for the highly sorbed odorant.

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Differential Expression of Odorant Binding Protein Classes in the Olfactory Epithelium of the hawkmoth *Manduca sexta*: Histological Localization.

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JOHN T. JONES (University of South Carolina)
KIMBERLY D. LOMMAN (University of South Carolina)

The olfactory epithelium of the hawkmoth *Manduca sexta* is organized into distinct domains or patches; the neuronal epithelium contains two domains containing either pheromone specific or general-odorant sensitive sensilla. Previous studies identified 3 classes of Odorant Binding Proteins (OBPs) that are differentially expressed in the antenna: Pheromone Binding Protein (PBP) is male specific and associates with the long pheromone specific sensilla while 2 classes of General-Odorant Binding Protein (GOBP1 and GOBP2) are expressed in both female and male antennae and associate with the short general-odorant sensitive sensilla (Vogt *et al.*, 1991, *J. Neurobiol.* 22:74 & *J. Neurosci.* 11:2972). These studies indicate that respective OBPs serve as molecular markers of the olfactory domains. We have now employed immunocytochemical and tissue *in situ* approaches to document the patterns of expression of the respective OBPs within the antenna. These studies show that PBP expression is restricted to the domains of the long pheromone specific sensilla, while expression of both GOBPs is restricted to the domain of short general-odorant sensitive sensilla. Each sensillum includes several olfactory neurons and three support cells. Immunocytochemical analysis indicates that PBP protein is present in both the tormogen and trichogen support cells; however, *in situ* analysis indicates that PBP mRNA is only present in the trichogen support cell; this observation suggests that PBP may be expressed in only one cell type (trichogen), secreted into the lumen of the sensillum and then cycled back again into either both or a different cell type (tormogen). This further indicates significantly different functions for the respective support cells. These studies form a framework for examining how the determination of fate, established at the onset of development, is translated through gene regulation into the functional organization of the olfactory epithelium.

Research has been supported by NIH-DC00588.

Odorant Confusion Matrix Response Patterns: Relation To Medical History. DANIEL B. KURTZ, PAUL R. SHEEHE, PAUL F. KENT, DAVID E. HORNUNG, THERESA L. WHITE, STEVEN L. YOUNGENTOB, JAMES E. SCHWOB, MAXWELL M. MOZELL, ELIZABETH M. BELKNAP, and PRECHA EMKO (Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, NY 13210).

The Odorant Confusion Matrix (OCM) has been an effective indicator of olfactory dysfunction in that it accurately reflects hyposmia consistent with reports of olfactory loss. In a retrospective study, differences in the OCM response patterns for 184 hyposmic patients were evaluated against medical histories. Dissimilarities between any two OCM patterns were quantified by a measure based on information transmitted resulting in a 184x184 triangular dissimilarity matrix. Dissimilarities were fit to a 5-dimensional space by multidimensional scaling (SYSTAT) such that patients with similar OCM patterns were closer together in space than people with different OCM patterns. To address the possible relation between OCM response patterns and medical histories, a step-wise regression was performed relating position in 5-dimensional space to 32 demographic and medical history variables. The effect of these variables on the PATTERN of response, as opposed to overall performance (percent correct), was evaluated by regressing out overall performance prior to a step-wise regression procedure. Three variables were found to be significantly related to the pattern of resonse (position in 5-space) and independent of degree of hyposmia, viz., patient age at the time of olfactory loss, recent head injury, and past chemical exposure. Subjects from another study on colds (see poster by Chojnacki et al., 1994), when added to this analysis, produced unique patterns of response (position in 5-dimensional space) despite a wide range of hyposmias. While it is likely that the interaction between mucosal solubility and odorant identification forms the basis for the OCM patterns resulting colds, identification of the other response patterns remain to be identified. These data suggest that some disease processes may produce distinct alterations in odorant coding which are manifested by unique odorant confusions which can be illuminated by the OCM.

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Chemosensory (Olfactory) Event-related Potentials in Patients with Dementia Associated with Parkinsonism. W. JAMES EVANS, LIYING CUI, and DANIEL D. TRUONG (Department of Neurology, University of California, Irvine).

Olfactory event-related potentials were recorded from five demented patients (age 71-81 years) with parkinsonian features not responsive to dopaminergic therapy. Diffuse Lewy body dementia was diagnosed in three patients and progressive supranuclear palsy was diagnosed in two. Severity of dementia ranged from mild to severe with patients scoring between 6 and 29 on the Mini-Mental State Examination. UPSIT scores ranged from 11 to 36. Detection thresholds for amyl acetate were elevated in all but two patients, with the least demented showing normal sensitivity and the most severely demented patient being unable to comprehend the task. Evoked potentials were elicited by a monorhinal odorant stimulus consisting of 50% amyl acetate at 40 ms duration, 5 L/min flow rate and a variable interstimulus interval of 6-30 s. No behavioral response was required of the patients nor were they required to perform velopharyngeal closure. The electroencephalogram from three electrode sites (Fz, Cz, and Pz, referenced to A1) was amplified 20,000 times, filtered using a bandpass of 0.1-100 Hz and digitized at 125 Hz, resulting in a timebase of 4 s. A 15 Hz digital low-pass filter was also applied to enhance the primary components of the evoked potential. A triphasic waveform was observed at all electrode sites in all five patients. The peak latencies and amplitudes of the N1 and P2 components in this group of patients did not differ significantly from similar measurements in an age-matched control group. These findings contrast with the delayed peak latencies seen in most patients with Parkinson's disease responsive to treatment with levodopa and dopamine agonists.

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Topical Corticosteroid Treatment of Nasal/Sinus Disease Olfactory Loss. A. MOTT*, D. LAFRENIERE*, A. APTER*, C. SAMPSON. (University of CT Health Center, *School of Medicine.)

The purpose of this study is to establish the efficacy of topical corticosteroids (TCS) in the treatment of olfactory loss due to chronic nasal and sinus processes. Forty subjects (21 male, 19 female; mean age=49, range 24-72) from the CT Clinical Research Center (CCCRC) with nasal polyps, chronic sinusitis and/or chronic rhinitis were treated with 8 wks of a TCS (37 Nasalide, 3 beclomethasone dipropionate aqueous) in a head-down-forward (HDF) position. The TCS was preceded by 2-3 weeks of systemic antibiotics if sinusitis was judged to be present. Olfactory loss was gradual in 60% and sudden in 25%. Olfactory fluctuations were reported in 68%, distortions in 10%, and phantoms in 23%. Previous use of corticosteroids was reported by 48% (23% systemic; 35% TCS), and, of these, olfaction improved in 20%. A history of chronic nasal/sinus symptoms was reported by 85%; 33% facial pain, 73% post-nasal drip, 50% runny nose, and 63% nasal obstruction. Polyps were previously diagnosed in 40%, sinusitis (28%), asthma (10%), and aspirin sensitivity (13%). Previous polypectomy occurred in 35%, and sinus surgery in 25%. The CCCRC olfactory test measured a butanol threshold and odorant identification score for each side and provides an overall composite score (possible range: 0 [anosmia] to 7 [normosmia]). Pre-treatment testing confirmed severe hyposmia in 5 subjects and anosmia in 35. Baseline ENT examination was positive for mucosal thickening on each side in 75% of subjects. Polyps were seen in 70% of subjects: 43% above the middle turbinate, and 45% below. Minimal polypoidal mucosal change was seen in 10% (lt) and 20% (rt) and moderate to severe in 45%(lt) and 35%(rt). CT scanning of the paranasal sinuses was obtained in 35 subjects and sinus x-rays in 3. Positive findings (mucosal thickening, opacification, haziness) were seen in the: ant./middle ethmoids in 78% each side; frontals in 48%(lt) and 40%(rt); maxillary sinuses in 70%(rt) and 75%(lt); posterior ethmoids in 78%(rt) and 73%(lt); and sphenoids in 40%(rt) and 45%(lt). After treatment, composite olfactory scores changed from a mean of .56 (SD=.77) at baseline to 2.7 (SD=2.4) (paired t-test, p= <.0001). Positive response (increase in composite score of $\leq$ 3.0 in at least one nostril), was seen in 21/40 subjects treated. After treatment, 56% reported improvement in nasal breathing, 24% in facial pain, 40% post-nasal drip, and 54% runny nose. ENT examination showed less severity of polypoidal mucosal changes (Fisher's exact test: rt., p=.01; lt., p=.04). Severe nasal polyps were smaller in 44%, although changes in polyp size for the entire group did not reach statistical significance. These data verify improvement in olfaction, symptoms and ENT examination findings in subjects with severe or total olfactory loss and chronic nasal /sinus processes treated with TCS in a HDF position. Improvement in olfaction may precede nasal polyp reduction.

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Sensory- and Cognition-Based Olfactory Functioning in Huntington's Disease. STEVEN NORDIN (UCSD Medical Center and San Diego State University), CARLA VIAZCAN, KRISTI ACKERMAN (San Diego State University) and CLAIRE MURPHY (UCSD Medical Center and San Diego State University)

Previous research has demonstrated olfactory dysfunction in several types of dementia, including Alzheimer's disease, Parkinson's disease, Down's syndrome, and HIV dementia complex. However, far less is known about olfaction in Huntington's disease (HD). Therefore, in the present study a wide range of olfactory functions, from mainly sensory to cognition based, were assessed in patients with HD and in normal controls (NC) matched for age, gender, and education: absolute detection (butanol; ascending method of limits), intensity discrimination (butanol; staircase method), quality discrimination (match-to-sample paradigm), short-term recognition memory, and identification (UPSIT and the San Diego Child-Odor-Identification Test). Taste or vision were used as comparison modalities to determine whether a potential decline in a particular function was specific to olfaction per se, or whether it was more global in origin. The results showed significantly poorer olfactory performance in the HD patients than in the NC on all functions studied, except for recognition memory. No significant difference in performance between groups was found for the comparison modalities, suggesting that the poor olfactory performance in HD predominantly reflects actual olfactory dysfunction. The relatively spared short-term recognition memory in HD is not surprising since previous research on vision and audition has demonstrated that this function is less affected than other memory functions in HD. In conclusion, these findings, integrated with previous studies on olfaction and dementia, motivate further research on differences between various dementias with respect to various olfactory functions.

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G_s-Protein Deficient Pseudopseudohypoparathyroid Patients Evidence Normal Olfactory Function.
RICHARD L. DOTY, ALBERTO D. FERNANDEZ, DONALD A. McKEOWN (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA), MICHAEL A. LEVINE (Department of Medicine, Division of Endocrinology and Metabolism, Johns Hopkins School of Medicine, Baltimore, MD), and ARNOLD MOSES (Department of Medicine, State University of New York Health Science Center, Syracuse, NY).

In non-human vertebrates, three classes of guanine nucleotide-binding proteins are found in the olfactory epithelium: G_s (stimulatory), G_i (inhibitory), and G_o (other). One G-protein, G_{olf}, is the predominant G_s-protein in the cilia, suggesting it may be involved in the olfactory transduction process. Two observations derived from human studies provide indirect support for the view that G-proteins are involved in human olfactory transduction. First, the perceived intensity of odorants to humans positively correlates with the amount of adenylate cyclase (an enzyme closely associated with G-protein mediated events) induction in a frog ciliary preparation (Doty et al., 1990). Second, human subjects with Type IA pseudohypoparathyroidism (PHP-Type IA), a model of G_s protein deficiency associated with generalized hormone resistance, have decreased olfactory function. Individuals with Type IB PHP, a form of PHP unaccompanied by G_s protein deficiency, evidence normal olfactory function (Weinstock et al., 1986; Ikeda et al., 1988). We now report that patients with a rare variant of PHP-Type IA, namely pseudopseudoparahypothyroidsm (PPHP), also evidence G_s-protein deficiency, as observed in Type IA PHP patients, yet have no alteration in their ability to smell. Since PHP-Type IA patients evidence hormone resistance, whereas PHP-Type IB and PPHP patients do not, hormone resistance may be an important factor related to the observed olfactory dysfunction.

Doty RL, Kreiss DS & Frye RE, *Brain Res* 527:130-134, 1990.
Ikeda K, Sakurada T, Sasaki Y, Takasaka T & Furukawa Y, *J Laryngol Otol* 102: 1111-1114, 1988.
Weinstock RS, Wright HN, Spiegel AM, Levine MA, Moses AM, *Nature* 322: 635-636, 1986.

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Cross-adaptation of Sweaty-Smelling 3-Methyl-2-Hexenoic Acid by a Structurally-Similar, Pleasant-Smelling Odorant.

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Cross-adaptation, a potential measure of the degree to which odors share common sensory channels, is affected by perceptual similarity. How structural similarity, in the absence of perceptual similarity, influences cross-adaptation is unknown. The present study assessed cross-adaptation by structurally similar, but perceptually different, odorants; a 10:1 mixture of (E)- and (Z)-3-methyl-2-hexenoic acid (3M2H; a principal component of human underarm odor), and a 3:1 mixture of the (E)- and (Z)- ethyl esters of 3M2H (EE3M2H), which possess a pleasant, fruity odor. Magnitude estimates for 3M2H were decreased following adaptation to EE3M2H. Cross-adaptation was asymmetric; adaptation to 3M2H did not significantly affect the perceived intensity of EE3M2H. Further testing with the isolated (E)- and (Z)- isomers of EE3M2H as adapting stimuli demonstrated that the (E)- isomer more completely cross-adapted 3M2H than did the (Z)- isomer. By contrast, there was no significant cross-adaptation between 3M2H and the fruity-smelling ethyl esters of its homologues, 3-methyl-2-octenoic acid (EE3M2O) and 3-methyl-2-pentenoic acid (EE3M2P). Similarity ratings revealed no differences among the three ethyl esters in their perceptual similarity to 3M2H. These results demonstrate that structurally-similar, yet perceptually-distinct, odorants may cross-adapt and indicate that cross-adaptation may be affected not only by perceptual similarity, but structural similarity as well.

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Evidence For a New Perceptual Ability: Determining Which of Two Individual Scents Is on Top.

ROBERT E. JOHNSTON (Cornell University)

Most mammals communicate by scent marking; a common pattern is that one individual marks in the same places that other individuals of the same species have marked. What information can a third animal obtain from a place that has been marked by two other individuals? In a large series of experiments using habituation methods, we have discovered that golden hamsters selectively remember the individual's scent that is on top, regardless of the spatial distribution of the two stimulus scents. These scents can be placed with one covering the other, in a crossed pattern, or even with some exemplars of the "bottom scent" not overlapped by the "top scent" - hamsters still selectively remember and respond to the scent of the individual that was on top, as long as at least one exemplar of the bottom scent was partially covered. These results suggest that hamsters detect the existence of two individual scents, determine which one is on top, and selectively remember this top scent. How hamsters determine which scent is on top is not presently known, but this ability is likely to be a general one in mammals because it has obvious potential benefits: One animal can focus attention on the most recent other individual that has deposited scent in an area. As far as we are aware, this sophisticated perceptual ability has never been described or even suggested before; it has implications for theories of mixture perception, individual recognition by scent, olfactory communication, and for more general ideas about the kinds of perceptual strategies and abilities employed by animals.

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Empirical Test of Models of Odor Interaction: MATS J. OLSSON, WILLIAM S. CAIN, & FRANC T. SCHIET (John B. Pierce Laboratory and Yale University).

In recent years, various mathemathical models have been suggested for prediction of odor intensity of mixtures from either the concentration or perceived intensity of components. Despite some empirical evaluation of the different models, no consensus has been reached on which to prefer. In this study, three binary mixtures were investigated for perceived intensity and quality. Five models of odor interaction were evaluated: Strongest Component, Vector, U, UPL2, and Equiratio Mixture (ERM) model. The three binary mixtures investigated were linalyl acetate paired with cineole, with geraniol, and with hexyl salicylate. Substances were mixed in the vapor phase from adsorbent sources and presented in squeeze bottles. Subjects rated perceived intensity in relation to a standard by means of a visual analogue scale (vas). The quality of a mixture was given as rated similarity (vas) to the quality of either of its components. With respect to perceived intensity of mixtures, the results exhibited the same type of pattern frequently seen before. The perceived quality of mixtures depended on the perceived intensity of the components. For instance, the quality of a mixture was equally similar to both its components when the components, as presented separately, were equally strong. All models performed reasonably well in terms of correlational fit. The ERM and UPL2 models that predict mixture intensity from information about the psychophysical functions of components typically overestimated mixture intensity. Nevertheless, it is argued that the general principles of mixture additivity are reflected in the power function that typically describes the psychophysical relation for single substances. This would mean that the addition of two different substances, on the one hand, and the addition of a single substance to itself, on the other, would share common principles. However, additivity in mixtures seems to be lower than the additivity of single substances that constitute the mixture. Accordingly, a Power Function Model, including the estimation of an exponent (n) for the mixture, is proposed where the mixture intensity (Rab) relates to its component intensities (Ra and Rb) such that $Rab = (Ra^{1/n} + Rb^{1/n})^n$. The model clearly is as successful as any of the others in the prediction of odor intensity of mixtures.

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Olfactory Dysfunction in the Elderly: Identification of Potential Precursers CLAIRE MURPHY (San Diego State University and UCSD Medical Center)*, JILL RAZANI (San Diego State University), TERENCE M. DAVIDSON, ALFREDO A. JALOWAYSKI (UCSD Medical Center)

One of the major objectives of the San Diego Longitudinal Chemosensory Aging Study is to quantify the extent of chemosensory impairment over the life-span in a normally aging population of 100 people. Past research in the field has employed a variety of populations in order to assess age effects on olfactory function. Clinical work over the past decade has highlighted the incidence of olfactory impairment as a result of nasal sinus disease, allergic rhinitis, inflammatory processes, and head trauma. Furthermore, olfactory dysfunction has been documented in a number of dementias (e.g., Alzheimer's Disease, Parkinson's Disease), which tend to be most common in later life. Even subjects who are at risk for dementia tend to show impairment in olfactory function. We hypothesized that the pure effects of aging itself on olfactory function were somewhat overestimated in earlier studies, including some of our own, because elderly subjects were included without regard to their nasal sinus health and without regard to their dementia status. Among the inclusion criteria for the present study were normal results on a complete ENT examination, including a history, nasal cytology, rhinomanometry, and nasal endoscopy; as well as scores within the normal range on a standard neuropsychological scale of dementia status. Thresholds were assessed for both nostrils using a two-alternative, forced-choice procedure with butanol as the odorant. Analysis of Variance showed the subjects to be less sensitive as a function of age, as we and others have reported in previous studies. The difference in olfactory function between the elderly and younger subjects in the present study was substantially smaller than in previous studies in which subjects were not assessed for nasal disease or dementia. The present results thus suggest cause for optimism: Older people do, indeed, show poorer olfactory function than younger people do, but, the degree of impairment in the normal, healthy, elderly person is less than previous estimates based on heterogeneous groups of elderly persons; and we are coming to understand the additional factors that precipitate more dramatic decline in olfactory function in subgroups of older people.

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Genome analysis of human olfactory receptors: gene clusters, diversity and individual variation. D. LANCET, N. BEN-ARIE, U. GAT, S. HORN-SABAN, M. KHEN, M. NATOCHIN, E. NEKRASOVA & N. WALKER (Dept. Membranes & Biophys.,Weizmann Institute Rehovot, Israel).

Olfactory receptors (ORs) constitute a superfamily of hundreds of G-protein coupled receptors, as also predicted by a Receptor Affinity Distribution (RAD, PNAS 90:3715-3719 (1993)). We cloned 16 OR genes in a 0.35Mb genomic cluster on human chromosome 17 (Ben Arie et al. Human Molec. Genet., in press), belonging to 4 different gene subfamilies. We suggest that the OR repertoire has evolved by repeated duplication of an entire gene cluster, on several chromosomes (Current Biol. 3:668-674 (1993)). Gene conversion may play a role in the generation of OR diversity. Data on a new cluster we identified on human chromosome 11, containing >12 OR genes, are used to validate these concepts. The genetics of specific anosmia show large individual differences, probably stemming from OR gene polymorphisms, and consistent with a "threshold hypothesis", whereby threshold is determined by the highest affinity OR. Candidate OR allele pairs are seen at two of the gene loci on chromosome 17, possibly related to the expected polymorphism. We are currently expressing human genomic and cDNA OR clones, which, together with methods for detecting point mutations, could allow genotype-phenotype correlation, and a better understanding of OR specificity, diversity and variation.

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THE GENOMIC ORGANIZATION AND REGULATION OF OLFACTORY RECEPTOR GENES

RANDALL R. REED[1], KAREN A. SCHRADER[1], ANN OLSEN[2] ANNE M. CUNNINGHAM[1], ROBERT Y-L TSAI[1], PANKAJ QASBA[1], IRENE C. GRIFF[1]. (Howard Hughes Medical Institute and Department of Molecular Biology, Johns Hopkins School of Medicine)

The ability of mammals to discriminate between a large number of odorant stimuli is thought to arise in part from the differential expression of olfactory receptor genes by the sensory neurons. Using antibodies directed against sequences from the highly divergent carboxyl terminus of we have demonstrated that the putative receptor proteins are highly enriched in the dendritic knobs and cilia of the sensory neurons. Moreover, only a small fraction of the sensory neurons express any particular receptor protein. We have recently undertaken a series of transgenic mouse experiments to elucidate the specific sequences that are required to direct and restrict the expression of a receptor gene to a subset of the receptor neurons. The genomic organization of these receptors in the genomes of mouse and human has been examined in our laboratory. The receptor genes exist in approximately 500 copies and are distributed on several chromosomes. Interestingly, they display similar linkage patterns to non-receptor genes in the two species suggesting an evolutionary conservation of organization and perhaps regulation. Detailed analysis of the receptor genes in mouse and human have revealed that highly related receptors are located in close proximity in the genome. Unequal crossing-over between homologous receptors would lead to the loss of a portion of the receptor repertoire and may explain the origins of specific anosmias. Experiments are currently underway to look for variations in the receptor repertoire of humans and mice and to correlate these with sensory deficits.

Supported by the Howard Hughes Medical Institute

Expression of Olfactory Receptor Chimeras. TIMOTHY S. MCCLINTOCK and MICHAEL R. LERNER (Yale University School of Medicine).

Replacing the intracellular loops which mediate coupling to G-proteins in the human B2-adrenergic receptor with the corresponding loops from rat olfactory receptors created chimeras which stimulated adenylyl cyclase in response to B-adrenergic agonists in melanophores. While chimeras in which only the third loop was replaced did not allow functional expression, those containing the second and third, third and carboxy terminal domain, or second, third and carboxy terminal domain from olfactory receptors were functional. These data indicate that olfactory receptors are capable of activating G-proteins in melanophores. Expression of a B2-adrenergic and an olfactory receptor which were mutated to contain an epitope tag at the amino terminus (extracellular domain) showed different patterns of immunostaining. In unpermeabilized melanophores, the tagged B2-adrenergic receptors showed staining at the plasma membrane while the olfactory receptor did not. This suggests that the processing and sorting of olfactory receptors is significantly different from the 20+ members of the G-protein coupled receptor family previously expressed in melanophores.

Identification of Olfactory Genes by Single *P*-element Mutagenesis of Inbred *Drosophila melanogaster*. ROBERT R. H. ANHOLT (Depts. of Zoology and Biochemistry, North Carolina State Univ., Raleigh, NC 27695), RICHARD F. LYMAN and TRUDY F. C. MACKAY (Dept. of Genetics, North Carolina State Univ., Raleigh, NC 27695)*

We have constructed highly inbred lines of *Drosophila melanogaster* that differ only in the location of a single, stably integrated, marked *P*-element construct (*P[lArB]*). To determine whether any of these randomly inserted *P*-element constructs disrupt genes affecting olfaction, we subjected 82 lines that contain the *P*-element on the second chromosome to a simple, rapid and highly reproducible behavioral assay in which the avoidance response of flies to benzaldehyde was monitored. Using this assay we identified 7 lines with aberrant olfactory behavior. Flies from these lines tend to avoid benzaldehyde, but their responses are weak and appear delayed. The *P[lArB]* construct contains a β-galactosidase reporter gene that has a promoter, but lacks an enhancer. Insertion of this construct near the enhancer of the affected gene will enable expression of the reporter gene in tissues where the mutant gene is normally expressed. Examination of the 7 mutant lines identified in our behavioral assay showed that 5 of these lines expressed the reporter gene in the third antennal segment, one of the main olfactory structures of *Drosophila melanogaster*. In three of these lines expression of β-galactosidase appears limited to this organ. Two lines show staining in the second antennal segment and the maxillary palps as well. One mutant line did not reveal detectable staining in adult flies, but showed highly localized and intense staining in larval antennal organs. In contrast to the staining patterns of lines deficient in olfactory responsiveness, only 1 of 6 *P*-insert lines with normal olfactory behavior expressed β-galactosidase staining in the third antennal segment, but only weakly. Isolation and characterization of *P*-element tagged genomic DNA sequences from our behavioral mutants will enable us to correlate expression of identified gene products, including odorant receptors, with a behavioral response to one well-defined odorant.

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Expression of Odorant Receptor Proteins During Postembryonic Development in Zebrafish. CHRISTINE A. BYRD (University of Virginia), RICHARD G. VOGT (University of South Carolina), and PETER C. BRUNJES (University of Virginia).

Zebrafish represent an outstanding model for examining the correlation between ontogeny of the olfactory system and ontogeny of chemosensory-based feeding behaviors. Hansen and Zeiske (J. Comp. Neurol. **333**, 1993) have described the postembryonic development of the olfactory epithelium: zebrafish hatch three days post-fertilization with a simple olfactory placode, and the olfactory rosette begins forming around 3 weeks, becoming fully formed by 6 weeks. In an initial study, we have isolated three putative odorant receptor genes from zebrafish and have used these in *in situ* hybridization analyses to examine the timing and patterning of expression of receptors. PCR primers were designed to encode conserved regions of catfish olfactory receptors (Ngai et al., Cell **72**, 1993) and used to amplify homologues from genomic DNA isolated from adult zebrafish. Products were cloned, sequenced and characterized against the sequences of known G-protein coupled receptors; the highest similarity was with olfactory receptors. To examine the distribution and ontogeny of expression of the mRNAs from these putative odorant receptors, *in situ* hybridization experiments were performed with digoxygenin-labelled probes using standard protocols. When a mixed probe was used, labelled cells were seen scattered throughout the sensory epithelium of the olfactory rosettes in adults. A similar expression was seen in three-month old fish. The location of the labelled cells is consistent with the hypothesis that they are olfactory sensory cells; this supports the evidence that these clones are odorant receptors. We are currently looking at younger animals to detect the onset of receptor expression. In addition, we are examining the distribution of the three probes in an attempt to find differential patterns of expression.

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Bradykinin Induces Attachment Of The Barnacle Balanus Amphitrite (Darwin). MARION MCCLARY, JR. (Duke University Marine Laboratory).

Barnacles reproduce sexually by pseudocopulation. Pseudocopulation is followed by the brooding of embryos and the release of larvae that pass through a series of naupliar stages to a cyprid attachment stage. Peptides that end with arginine or lysine mimic pheromones that induce cyprids to attach near adults of their species (Tegtmeyer and Rittschof, 1989, Peptides 9:1403-1406; Pettis, 1991, Doctoral dissertation, University of North Carolina, Chapel Hill. 139 pp.; Rittschof, 1993, Amer. Zool. 33(6):1). The mechanism of pheromone detection is unknown. Bradykinin, a nonapeptide, has the sequence of a pheromone mimic. Cyprids, less than a day old, were exposed to 10^{-8} M bradykinin in a flow of 6.8 ml/min for 10 mins, and under static conditions at 10^{-8} and 10^{-9} M for 22 hrs at room temperature. Bradykinin induced settlement behavior and caused the larvae to initiate metamorphosis. Initial studies with antibodies to bradykinin suggest bradykinin binding sites are on the larval attachment organ. Additional work is necessary to confirm this interpretation.

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Reiterative responses to single strands of odor promote sustained upwind flight and odor source location by moths NEIL J. VICKERS AND THOMAS C. BAKER (Dept. of Entomology, Iowa State University, Ames, Iowa 50011

We characterized single upwind surges of flying male *Heliothis virescens* moths in response to individual strands of pheromone generated experimentally in a wind tunnel. We then showed how this surge functions in this species as a basic 25-cm, 0.35-sec-long building block that is strung together repeatedly during typical male upwind flight in a normal pheromone plume. The template for a single iteration, complete with cross-wind casting both before and after the straighter upwind surging portion, was exhibited by males flying upwind to pheromone and experiencing filament contacts just frequently enough to produce successful upwind flight to the source, as hypothesized by an earlier model. Also as predicted, when filament contact by males became more frequent, only the straightest upwind portions of the surges were reiterated, producing direct upwind flight with little cross-wind casting. In-flight electroantennogram recordings (EAGs) made from males in free-flight upwind in a pheromone plume from a normal point source further support the idea that a high frequency of filaments encountered under the usual pheromone plume conditions promote only these repeated straight surges. In-flight EAGs also showed that when filament contacts cease, the casting, counterturning program begins to be expressed after a latency period of 0.35 sec. Taken together these results provide a plausible explanation for how male and female moths, and perhaps many other insects, fly successfully upwind in an odor plume and locate the source of the odor, using a surging-casting, phasic-tonic response to the onset and disappearance of each odor strand.

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A field test of the influence of odors from conspecifics on den selection in the spiny lobster *Panulirus argus*. N. DEAN PENTCHEFF (Biological Sciences, Univ. of South Carolina). GABRIELLE A. NEVITT (Institute for Neurosciences, Univ. of Oregon). KENNETH J. LOHMANN (Dept. of Biology, Univ. of North Carolina). RICHARD K. ZIMMER-FAUST (Biol. Sciences, Univ. of South Carolina).

The olfactory abilities of the Florida spiny lobster (*Panulirus argus*) have been intensively studied as a model system, but most behavioral work concerning lobster olfaction has concentrated on odors associated with food or prey items. Based on field and lab studies, spiny lobsters are known to live in aggregations, preferentially choosing to live with conspecifics. We performed a field investigation of the possibility that this social behavior is mediated by odor cues. We constructed artificial dens inserted in cavities in the reef to mimic natural den sites. Using a submersible perfusion system, each den was continuously perfused either with ambient seawater drawn past remotely located lobsters or with unaltered ambient seawater. Each day during the two week experimental period, the dens were censused, new recruits were removed, and the odor treatments were switched. Our results show that lobsters preferentially recruit to dens perfused with the odor of conspecifics. As expected for an odor cue promoting aggregation, the cue is transient, lasting less than a day following its application to a den.

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Measuring Olfactory Stimulus Samples of Lobsters Orienting in a Turbulent Plume.

JENNIFER A. BASIL AND JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole MA 02543)

Lobsters , Homarus americanus , are capable of navigating to distant odor sources, relying on their lateral antennules to make directional choices in turbulent odor plumes. Three different stages of orientation behavior are apparent in the lobsterÆs search patterns within the plume. At initial contact with the plume, animals decrease their walking speeds and heading angles. In intermediate positions within the plume walking speed and heading angle remain constant, and as they near the odor source, walking speed decreases along with heading angle (Moore, Scholtz, and Atema, 1991). These search patterns indicate that they are capable of extracting both directional and distance information from cues contained within the plume. Measurements of the fine scale three dimensional structure of turbulent odor plumes shows spatial gradients of features such as pulse height, onset slope, and distribution ,which could provide directional cues to orienting animals (Moore and Atema, 1991; Basil, Voigt, Mountain, and Atema, unpubl. data).The purpose of the present study was to implement techniques from which we can determine whether animals extract and use fine scale features to successfully navigate to a distant odor source. We thus measured the bilateral temporal intensity patterns encountered by a lobster with stimulus recording micro-electrodes mounted on, and positioned directly over the lateral antennules of , freely orienting lobsters. This electrochemical microelectrode technique allowed us to measure concentration changes of an odor tracer with spatio-temporal resolution comparable to chemoreceptor organs. To examine how lobsters may extract information using bilateral chemoreceptor organs, a submersible, portable amplifyer to which up to 2 electrodes could be connected was mounted on the carapace of a freely orienting lobster. This "backpack" transmitted the tracer encounter rate to a computer with software (IVEC, Med Systems) designed to convert the signals to real-time odor concentration measurements. The simultaneous video record of the lobster's path was digitized and analyzed to determine heading angle, turning angle,and walking speed. This record was synchronized with the IVEC trace during filming so that instantaneous bilateral odor plume characteristics and the behavior of the orienting lobster could be correlated. These techniques allow us to infer how animals extract and use information from odor plumes to navigate to distant odor sources. Supported by NSF IBN 9212650

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How blue crabs (*Callinectes sapidus*) find their prey in nature: I. Measurement and modeling of odor plumes in turbulent flows.

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Many predators locate their food using olfaction, based on odors emanating from their prey. In estuarine environments, water flow is turbulent and yields odor plumes with a complex internal structure of chemical concentrations. We are investigating the mechanisms by which blue crabs are able to follow an odor plume by modeling the characteristics of naturally-observed odor plumes, then testing the important parameters of those models against observations of crab behavior in nature. Simple plume models (Gaussian) draw an analogy between molecular diffusion and turbulent dispersal, yielding predictions that spatially or temporally average fine scale structure. More complex models can explicitly include the formation of fine scale structure through turbulence. To construct models of the odor plumes present in natural estuarine flows, we performed a series of field experiments. Flow velocities were measured to generate realistic descriptions of benthic boundary layer fluid mechanics (both mean flow and turbulence); high-speed electrochemistry was used to directly measure the distribution of fine scale chemical concentrations; video recording of dye plumes followed by frame-by-frame analysis was used to make synoptic plume measurements; and, fluorometry of dye tracers was used to measure time-averaged plume parameters. These physical data allow us to parameterize and validate models of odor plumes for the estuarine setting.

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How blue crabs (*Callinectes sapidus*) find their prey in nature: II. Mechanisms of orientation in turbulent odor plumes.

Blue crabs are important predators in estuarine environments. One means by which crabs locate food is by following odor plumes released by potential prey. Since water flows in natural estuarine habitats are turbulent, odor plumes have a complex internal structure. Hence, it is possible that crustacean predators may use information coded in the pattern of fine scale concentrations to determine distance or direction to prey items. We are investigating the mechanisms by which crabs are able to follow these plumes by coupling empirical measurements and modeling of natural plume structure with behavioral observations of crab responses to odor plumes in field habitats. In field studies, we tested crab responses to a number of odor sources based on natural prey items (clams, mantle fluid of clams, amino acid mixture comprising clam mantle fluid, and control treatments). Crab behavior relative to the odor plumes was video recorded and subjected to frame-by-frame analysis. Since physical and chemical measurements were coordinated with the observations of crab behavior, we were able to compare behavioral responses against the chemical distributions predicted by plume models. Hydrodynamically-based plume models can incorporate differing degrees of spatial or temporal averaging of the complex concentration structure. By testing different plume model predictions against observed crab behavior, we are able to assess the degree to which fine scale aspects of odor plume structure are important to crabs following an odor plume.

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Regulation of Gender-Specific Chemosensory Behaviors in Fiddler Crabs: Electrophysiological Properties of Male and Female Chemoreceptor Neurons.

MARC WEISSBURG and C. DERBY (Biology Dept., Georgia State University, Atlanta, GA.)

Fiddler crabs display pronounced gender-specific behaviors to compounds which stimulate feeding. Feeding behavior is regulated, at least partially, by chemosensory input from the feeding claw. Females show greater behavioral sensitivity than males, having higher feeding rates at equivalent food concentrations, and feed at low food concentrations that fail to elicit feeding in males. Gender-specific differences in electrophysiological responses were assayed from single unit extracellular recordings from chemoreceptor neurons in the claw and leg of two closely related species of fiddler crabs, *Uca pugnax* and *U. pugilator*. Test chemicals were 2 different mixtures of sugars and amino acids known to excite feeding behavior. Based on an analysis of 12-15 cells of each sex and species, response properties of cells on claws are also gender-specific, and consistent with behavioral patterns. In response to single presentations of test stimuli, female cells respond to lower concentrations than male cells, are active over a greater range of concentrations, and show generally increased firing rates relative to male cells tested at equivalent doses. Patterns of adaptation failed to show evidence of gender-specificity. Cells began to adapt to a continuous stimulus after 10-15 s regardless of gender. After a 60 s stimulus pulse both male and female cells appeared to disadapt fully after 3 minutes, and repeated presentations of the stimulus at 60 s intervals elicited little, if any, evidence of cumulative adaptation. The role of chemoreceptors on the dactyls of walking legs is uncertain, although these sensilla may contribute to patterns of orientation and/or regulation of feeding. We are currently collecting and analyzing data from cells on walking legs to determine if dose-response functions, or patterns of adaptation also exhibit gender-specificity. In addition, because legs are exposed to stimuli more frequently and possibly for greater intervals than are claws, leg and claw chemoreceptors experience different stimulus environments. Our study may also indicate whether there is a functional association between basic physiological response properties and the chemical stimulus environment experienced by receptors located on different appendages.

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Perception of Odor Mixtures by the Spiny Lobster: Influence of Mixture Interactions on Behavior. WILLERT LYNN, ELIZABETH MEYER, CECIL PEPPIATT AND CHARLES DERBY (Dept. of Biology, Georgia State University)

We have used the spiny lobster Panulirus argus in conditioning experiments to examine behavioral discrimination of adenosine-5'-monophosphate (AMP), betaine, L-cysteine, and their binary mixtures. Our results show that spiny lobsters can clearly discriminate among binary mixtures and their components: lobsters that were aversively conditioned to avoid responding to a binary mixture continued to respond to that mixture's components, and lobsters that were aversively conditioned to avoid responding to an odorant compound tended to continue to respond to binary mixtures containing that compound. Previous electrophysiological results have shown that when mixed, AMP, betaine, and L-cysteine produce nonlinear responses (i.e., mixture interactions) in members of a population of olfactory receptor neurons. A prior behavioral study using a different set of odorants -- AMP, L-glutamate, taurine, and their binary mixtures -- showed that those combinations of compounds that show mixture interactions in a population of olfactory receptor neurons also are most clearly behaviorally discriminated from their components. Together, these electrophysiological and behavioral results support the idea that peripherally generated mixture interactions in olfactory receptor neurons can enhance the contrast between the neural responses to mixtures and their components, leading to enhanced perceptual differences between these stimuli.

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Novel Water, Thyroxine, and the Timing of Olfactory Imprinting in Coho Salmon (*Oncorhynchus kisutch*).
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Pacific salmon are well known for their ability to learn odors associated with their natal stream as juveniles and later use these retained odor memories as adults for orientation during their homing migration. Experimental evidence suggests that olfactory imprinting by salmon occurs during a sensitive period associated with surges in plasma thyroxine (T4) levels during smolting. Life-history studies, however, suggest that imprinting may occur prior to smolting. A possible resolution of this paradox may lie in the finding that exposure of coho salmon smolts to novel water sources induced transient increases in plasma T4 levels. If novel water-induced T4 surges occur prior to smolting and T4 surges are required for olfactory imprinting, juvenile salmon experiencing novel water sources as they move through their watershed may learn olfactory waypoints (e.g., stream confluences). To test this hypothesis, we exposed juvenile coho salmon to the artificial odorant PEA or distinct water sources at different developmental stages and examined their ability to learn and respond to these odors as homing adults. We also examined whether exposure to these novel waters would elicit plasma T4 surges at distinct developmental stages. Our results indicated that mature coho salmon recognize and respond behaviorally to odors they have been exposed to for as little as 10 days as juveniles, particularly during smolting. However, these salmon did not demonstrate dramatic increases in plasma T4 levels during smolting and exposure to novel water sources had no effect on basal T4 levels. These results suggest that surges in plasma T4 levels during smolting may not be necessary for olfactory imprinting and accurate homing.

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Classically conditioned skin potential changes as a behavioral measure of olfactory response in the tiger salamander. K.M. DORRIES, J. WHITE and J.S. KAUER (Neuroscience Program, Tufts/NEMC, Boston, MA 02111)

The tiger salamander (*Ambystoma tigrinum*) is a commonly used species in vertebrate olfactory research. Our ability to integrate and interpret the growing body of anatomical, molecular biological and physiological information available for this species, however, is limited by the paucity of behavioral data in the tiger salamander for commonly used experimental stimuli. We describe here a method for measuring behavioral responses to olfactory stimuli in the salamander. Using classical conditioning, we have trained skin potential changes, an unconditioned response to a mild electrical shock, to occur in response to odor stimulation. Animals are immobilized with curare while skin potential is recorded with 2 surface electrodes contacting the forelimbs. A bipolar electric shock (0.1 - 1 mA) is applied to the tail, paired temporally with the termination of a 5 sec odor presentation. After training, salamanders respond to odor stimulation, but not to air, with a transient 50 - 500 mV change in skin potential. We have trained skin potential changes to amyl acetate at concentrations used in physiological studies (10^{-1} - 10^{-2} % vapor saturation), and can elicit responses to concentrations as low as 4 X 10^{-3} % vapor saturation. The response can be trained, extinguished and retrained in individual animals in a single several hour session. We are currently using this method to replicate results from prior behavioral studies that used a conditioned avoidance paradigm (Mason, Stevens and Rabin, 1980; Mason and Stevens, 1981). The skin potential response does not involve animal movement and thus has the potential to be used simultaneously with *in vivo* physiological recording. This method will be useful for determining the tiger salamander's ability to detect and discriminate a wide variety of stimuli at the behavioral level.

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Dimethyl disulfide (DMDS) - a sex attractant pheromone in golden hamsters? Lack of sex differences in attraction to DMDS and lack of androgen control of attraction to DMDS in males.

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ROBERT E. JOHNSTON (Cornell University)

Vaginal secretions of female hamsters are the source of odors that are highly attractive to males. Dimethyl disulfide (DMDS) has been identified as one component of these secretions that is attractive to males in very small amounts. If DMDS has specialized signal function, it should be more attractive to males than to females and the responses of males should be influenced by gonadal hormones. We tested these hypotheses in two experiments. In the first experiment we examined the attraction of males and females to odors in an neutral arena; the odors were vaginal secretions, DMDS, and a mixture of 3 mercaptans that have been suggested to have sexual functions. Males spent more time than females investigating vaginal secretion odors, but there was no sex difference in investigation of either DMDS or the mercaptans. In the second experiment males responses to the same three odors were investigated after castration and then after replacement therapy with capsules containing testosterone or capsules that contained no hormone. Hormone manipulations changed the responses to vaginal secretions but not to either DMDS or to the mercaptan mixture. These results suggest either that DMDS has no special significance as a sexual attractant in hamsters or that it only has such a special significance when in combination with other components of the vaginal secretion. In either case, however, the idea that DMDs is a specialized communicatory signal (a classical pheromone) seems to be incorrect.

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Person Identification: Can Dogs Match-to-Sample Scents from Different Sites on the Skin of Familiar and Unfamiliar People? W. J. CARR, MICHELLE GABLER, TINA MANWILLER, JUNE OSWALD, LANA RUSSECK & COURTENAY WALLACE (Beaver College)

Brisbin & Austad (1991) reported that three dogs trained on the American Kennel Club's Scent Discrimination Test (SDT) readily retrieved dumbbell-shaped items (DBs) hand-scented or elbow-scented by their handler (H), rather than DBs comparably scented by another person. But in generalization tests the dogs did not distinguish between H's elbow-scented DBs vs. the other person's hand-scented DBs, suggesting that they didn't react to H's person-specific scent regardless of the site from which it was taken. Since this report has been criticized (Sommerville et al., 1993; Toner & Miller, 1993) we conducted two studies using a Labrador Retriever that had learned the SDT and held the AKC Utility Dog title. It also learned a match-to-sample task (M-to-S), one person serving as the sample/match (S/M) and another as the lure (L), thus duplicating a task used with law enforcement dogs. The studies involved M-to-S training under same-site and cross-site conditions. Study 1 largely confirms Brisbin & Austad's report; with H as S/M and another familiar person as L, the dog M-to-S ($p < .05$) under two same-site conditions (S/M: hand/hand; L: hand) and (S/M: elbow/elbow; L: elbow) and two cross-site conditions (S/M: hand/elbow; L: elbow) and (S/M: elbow/hand; L: elbow), but not (S/M: hand/elbow; L: hand) or (S/M: elbow/hand; L: hand). Study 2 extends Brisbin & Austad's report; with two strangers serving as S/M and L, the dog M-to-S ($p < .05$) under one same-site condition (S/M: hand/hand; L: hand), but not (S/M: elbow/elbow; L: elbow). The dog also M-to-S ($p < .05$) under one cross-site condition (S/M: elbow/hand; L: hand), but not (S/M: hand/elbow; L: elbow). We infer: (a) cross-site M-to-S is more difficult than same-site M-to-S and (b) M-to-S is impaired if the S/M and L are strangers. Yet, law enforcement dogs are often used to M-to-S strangers' scents under cross-site conditions. We endorse Brisbin & Austad's view that such dogs be tested with proper controls for artifacts and shown to be sufficiently adept in person identification to warrant treating their responses as admissible evidence.

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A Novel Gland in the Nephropore of the Lobster, Homarus americanus: a Site for the Production of Chemical Signals? PAUL BUSHMANN and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

Urine serves in chemical communication in the lobster, *Homarus americanus*. The absence of male urine can delay the determination of male dominance (Karavanich C., and J. Atema. 1992. Biol. Bull. **181**:359-360.). Females can use male urine odor plumes to locate sheltered males (Bushmann, P., unpublished data), and males release urine in response to both male and female social encounters (Breithaupt, T. 1993. Biol. Bull. **185**:318; Bushmann, P. unpublished data). This study examined the nephropore area of sexually mature lobsters for the presence of a structure capable of producing a chemical signal. A mass of rosette glands, surrounding the ureter of all animals examined, was found. Morphologically each rosette appears generally similar to lobster tegumental glands, which produce phenoloxidase for the hardening of cuticle, but this nephropore gland differs from tegumental glands in several ways. All rosettes of this gland appear to empty their products into a large collecting duct, rather than each rosette possessing its own duct. The large duct terminates near the bladder, where there is no chitinous cuticle, suggesting a product other than phenoloxidase. Staining properties suggest that the nephopore gland is active at all times, whereas tegumental glands follow the molt cycle in activity. The granules in each rosette cell stain positive for tyrosine with the Millon reaction, indicating the presence of protein or peptide. Behavioral assays suggest that males respond differently to nephropore extract based upon dominance status. In summary, these glands seem to produce some novel proteinaceous product, and secrete this product into the urine. This product may be one of the urine components that affect lobster social behavior.

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Urine as a chemical signal in lobster dominance recognition.
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The maintenance of dominance hierarchies in the American lobster (*Homarus americanus*) is based on recognition of the dominant animal by the loser of a recent fight. Antennular chemoreception is essential for this recognition (Karavanich and Atema, Biol. Bull. 181: 359-360, 1992). Chronic catheterization has revealed that more urine is released in the presence of a second conspecific than during resting or during activity initiated by non-social disturbances (Breithaupt and Atema, Biol. Bull. 185: 318, 1993). We now report that urine. release is essential for the maintenance of dominance hierarchies in lobsters and that it is an integral element of aggressive behavior. Twenty pairs of males were fought for 20 min each on two consecutive days. In the first fight all lobsters were "pseudo-catheterized" , i.e. catheter tubes were attached but urine could still be released. For their second fight ten of the pairs (control) remained pseudo-catheterized, while the other 10 pairs (experimental) were completely catheterized, i.e. urine collected and not released into environment. During second fights, control subordinates immediately backed away from their previous dominant whereas experimental subordinates fought again for dominance. This result implies that urine is used for the recognition of the dominant animal from previous fights. In a second set of experiments urine production during fighting behavior was investigated and correlated with the behavior of the animal. Separate videocameras filmed the behavior of the lobsters and the rise of the urine level inside the scaled, transparent catheters. Lobsters regularly released urine when showing aggressive behavioral elements (as categorized by Scrivener, Fish. Res. Board Can. Tech. Rep., 235: 1-128, 1971). Defensive behavioral elements, however, were not accompanied by urine release. We conclude that aggressive lobsters transmit urine signals to their opponents and in subsequent fights these signals are used by a subordinate for the recognition of the dominant lobster.

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High Specificity of the Sea Lamprey Olfactory System to Four Classes of Bile Acids
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At the last annual meeting we reported that the olfactory system of adult sea lamprey (*Petromyzon marinus*) is highly sensitive to bile acids released by conspecific larvae. We also hypothesized that these compounds may function as migratory cues. The present study characterized the structure-activity relationships of bile acids as olfactory stimuli for adult sea lamprey and determined the number of olfactory receptor mechanisms for these compounds using electro-olfactogram recording. The relative potency of 38 bile acids indicated that three axial hydroxyls (OH), a 5α-proton and a sulfate ester at the 24-position or an unmodified carboxyl group were critical to the olfactory effectiveness of bile acids. These structural features characterize petromyzonol sulfate (PS) and allocholic acid (ACA), both of which are released by the larvae and are the most potent stimuli for adults. In addition, a sulfate ester at the 3-position increased the olfactory potency of bile acids lacking OHs. While taurine conjugation at the 24-position increased the potency of bile acids with one OH, it decreased the potency of bile acids with three OHs. Cross-adaptation experiments suggested that adult sea lamprey possess four types of bile acid receptor mechanisms which respond best to: (1) PS with a 24-sulfate ester and three OHs; (2) ACA with an unmodified carboxyl and three OHs; (3) taurolithocholic acid 3-sulfate with a 3-sulfate ester and no OH; (4) taurodeoxycholic acid with 24-taurine conjugation and two OHs. Binary mixture experiments confirmed this conclusion. These results further support the hypothesis that larval bile acids may function as a migratory pheromone for the adult sea lamprey.

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Isolation of Potential Musth-Alerting Signals from Temporal Gland Secretions of Male Asian Elephants (*Elephas maximus*), a new method.
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Temporal gland secretion is exuded by male Asian elephants (*Elephas maximus*) during their musth cycles, a period of heightened aggression. Light volatiles from this secretion may signal the male's close proximity to other elephants. Air-flow directed over collected samples of this secretion elicits a specific avoidance response by captive female Asian elephants. The avoidance response is apparently mediated by olfaction and consists of rapid movement away from the air-flow source, ear-flapping, and occasional vocalizations. A new procedure for sampling, storage, and analysis of the headspace of low concentrations (~ 1 ppm) of volatile compounds that may be components of conspecific chemical signals has been developed for temporal gland secretions. Modified from methods for measuring atmospheric trace gases, this method uses 850 ml internally electropolished stainless steel canisters. These canisters make it possible to store certain classes of volatile compounds, in stable form, for long periods of time prior to analysis or use in other experiments. The efficacy of this method was demonstrated by 1) the identical gas chromatographic / mass spectrometric identification of thirty compounds from TGS headspace samples at the time of collection and after storage for one year, and 2) the retainment of the biological activity over several years. During bioassays of a four female elephant group, two females consistently exhibited avoidance behavior. During the initial tests, the dominant female and a juvenile female, naive of male elephants, did not show avoidance. Subsequently, a year later, after exposure to males, this juvenile exhibited strong avoidance behavior, suggesting a learned component to this avoidance. In contrast, another, less volatile component of temporal gland secretion, cyclohexanone persistently evoked four types of bioresponses, especially flehmen, from most of the female elephants tested. The persistent response by female Asian elephants to cyclohexanone is the first time a single synthetic compound has elicited recurring bioresponses among elephants.

Supported by Biospherics Research Corporation

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Immunohistochemical Localization of Two Protein Precursors of the Axillary Odor.
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The characteristic odor which occurs in the human axillae has long been known to arise from the interaction of the cutaneous microorganisms with apocrine gland secretions. More recently, we have shown that the major component of the characteristic odor, 3-methyl-2-hexenoic acid (3M2H) is carried to the skin surface bound to water. soluble molecules in the apocrine secretion. These molecules appear to be two odor-binding proteins which we have designated apocrine secretion odor binding proteins 1 and 2: ASOB1 and ASOB2. Their apparent molecular weight, based on their mobility in SDS polyacrylamide gel is 45,000 daltons and 26,000 daltons, respectively. Both proteins bind 3M2H. Purified proteins from male subjects were used to develop highly specific polyclonal antibodies (raised in guinea pig. The antibodies were tested for cellular localization in specimens taken from two male and eight female volunteers. Samples were paraformaldehyde fixed, paraffin embedded and sectioned at 5 μm. Antisera and EZ-SEP-purified (Middlesex Scientific) immunoglobulin fraction of anti-ASOB1 and ASOB2, were tested at dilutions ranging between 1:100 to 1:4,000, for either 60 min. at room temperature, or 18 hours at 4°C. Specific immunoreactivity was noticed even at dilutions of 1:4,000 and it was localized only in the apocrine glands. Neither eccrine, nor sebaceous glands showed reactivity. No difference was noticed between male and female subjects.

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Proteinaceous Precursors to Human Axillary Odor: Apocrine Secretion Odor Binding Proteins.
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The characteristic odor which arises in the human axillary region consists of volatile C_6-C_{11} acids with the most abundant being (E)-3-methyl-2-hexenoic acid (E-3M2H). We have found that E-3M2H and other components of the characteristic odor can be liberated from the odorless, aqueous soluble components of apocrine secretion. The individual proteins found in apocrine secretions were separated, isolated and hydrolyzed with the resultant hydrolyzates analyzed by gas chromatography/mass spectrometry. This demonstrated that 3M2H was liberated from two proteins with apparent molecular masses of 26 and 45 kilodaltons; termed apocrine secretion odor binding proteins 1 and 2, respectively (ASOB1; ASOB2). Antisera to these proteins were prepared and used to examine a variety of body fluids from five male subjects. Several secretions (tears, nasal mucus, ear wax, serum and submandibular saliva, as well as areaolar and forehead sweat) contained an immunoreactive protein with the same electrophoretic migration pattern as ASOB1 (45 kD). Areaolar sweat and ear wax also demonstrated ASOB2-like immunoreactivity. Furthermore, the water soluble fractions of tears, saliva and forehead secretions from the same five subjects also contained 3M2H after hydrolysis. These results suggest (a) widespread occurrence of ASOB1-like proteins, albeit at low concentrations in several body fluids; (b) 3M2H may be bound to ASOB1-like proteins in these body fluids; and (c) 3M2H may contribute in some way to a variety of body odors and an individual's "odor signature."

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Paradoxical Suppression of Evoked Activity in Rat Olfactory Bulbs Slices by the Dopaminergic Antagonist, Haloperidol

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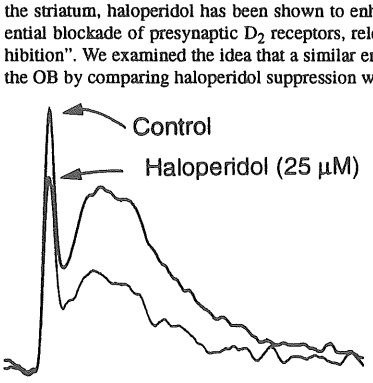
DAVID M. SENSEMAN (Div. of Life Science, Univ. of Texas at San Antonio)

D_1 and D_2 receptors for dopamine (DA) occur in the rat olfactory bulb (OB) with especially high amounts of D_2 receptors in the olfactory nerve layer. This suggests that dopaminergic periglomerular (PG) cells may inhibit presynaptically the flow of afferent odor information. We investigated this possibility by examining the role of DA agonists and antagonists in altering electrically-evoked activity in rat olfactory bulb slices stained with the voltage sensitive dye, RH 155, using multiple-site optical recording techniques. Bath application of the D_2/D_1 antagonist, haloperidol, at relatively low concentrations (10-100 μM) caused a significant, but reversible, dose-dependent decrease in the amplitude of the compound action potential (CAP) in the nerve layer as well as a reduction of the Ca^{2+}-dependent, slow depolarization in the glomerular layer. This result was unanticipated since DA blockade would be expected to increase, not decrease, evoked OB activity. In the striatum, haloperidol has been shown to enhance DA transmission by preferential blockade of presynaptic D_2 receptors, releasing DA neurons from "autoinhibition". We examined the idea that a similar enhancement might also occur in the OB by comparing haloperidol suppression with the effects of the DA agonists, apomorphine and PPHT. Like haloperidol, both DA agonists decreased the slow, Ca^{2+}-dependent glomerular depolarization. Neither agonist, however, significantly decreased the amplitude of the optically recorded CAP in the nerve layer making it less likely that haloperidol's effects result from increased DA release. Other possible mechanisms for this paraoxical suppression are currently under investigation.



Supported by: NIH/NIGMS GM 08194 and the San Antonio Area Foundation

CGP 55845, a $GABA_B$ Antagonist, Partially Reverses Glomerular Synaptic Depression in the Rat Olfactory Bulb Following Paired-Pulse Olfactory Nerve Stimulation

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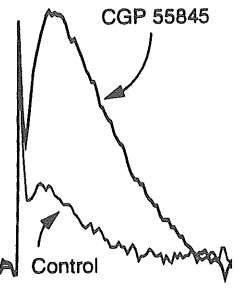
The pharmacology of paired-pulse synaptic depression in the glomerular region of the rat olfactory bulb (OB) was investigated in a slice preparation using multiple-site optical recording techniques. In both *in vivo* and *in vitro*, a conditioning shock to the olfactory nerve leads to a profound and long-lasting decrease in the efficacy of subsequent test shocks to evoke a neural response in the OB. Neurotransmitter(s) mediating this synaptic depression are not known although the $GABA_B$ agonist, baclofen, has been shown to exert a strong inhibitory effect on bulbar activity evoked by olfactory nerve stimulation and periglomerular (PG) cells in this region are GABA-ergic. We now report that CGP 55845, a $GABA_B$ antagonist, partially reverses paired-pulse synaptic depression recorded optically in the glomerular region of slices stained with the voltage-sensitive dye RH 155 (Fig. shows test response before and after 50 μM CGP 55845). The effect appears restricted to $GABA_B$ blockade since no reversal was observed with either antagonists of $GABA_A$ receptors (bicuculline and picrotoxin) nor with antagonists of dopamine D_1 and D_2 receptors (haloperidol and sulpiride). A small reversal was observed, however, after excitatory amino acid (EAA) blockade with DNQX, an antagonist of the kainate/AMPA receptor subtype.



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Receptive Fields of Sex-Pheromone-Responsive Neurons in the Antennal Lobes of the Sphinx Moth, *Manduca sexta*

THOMAS HEINBOCKEL, THOMAS A. CHRISTENSEN, JOHN G. HILDEBRAND (ARL Div. of Neurobiol., Univ. of Arizona, Tucson, AZ 85721)

In several modalities (e.g., vision, mechanosensation) sensory neurons respond selectively to input from spatially restricted areas of their environment. This information is either conserved or integrated in higher brain centers. Male moths use their antennae for detection of and orientation toward a sex pheromone-releasing conspecific female. Males must recognize the correct quantity and quality of the pheromone blend, but they also have to resolve the temporal and spatial structure of the stimulus at long and close distances to the female to ascertain the correct location of the odor source. In male *M. sexta*, axons of antennal sensory neurons specific to single components of the female's sex pheromone project to the male-specific macroglomerular complex (MGC) in the ipsilateral antennal lobe. The MGC comprises two major compartments, each of which receives input from sensory neurons "tuned" to one of the 2 essential sex-pheromone components. In the present study we use the moth antenna along its proximal-distal axis to study the receptive field organization of central neurons in the olfactory system.

Different regions of the antenna were stimulated consecutively with one of the 2 key pheromone components, or a mixture of both, while responses were recorded intracellularly from pheromone-responsive MGC projection neurons (PNs). When possible, cells were stained intracellularly with neurobiotin. MGC PNs generally fall into three groups with different receptive field properties based on their responses to a pheromone mixture: (1) neurons excited relatively independently of the region of the antennal flagellum stimulated; (2) neurons selectively excited by stimulation of the proximal region of the antennal flagellum; (3) neurons excited by stimulation of proximal and distal regions of the antennal flagellum but not its central regions. Neurons excited (i.e., showing increased firing) in response to pheromonal stimulation of particular antennal regions exhibited no decrease in background firing (i.e., were not inhibited) in response to stimulation of other regions.

MGC PNs can have arborizations in either one or both of the 2 compartments of the MGC and thus can respond to either one or both of the 2 essential pheromone components. The receptive field of a PN in response to a mixture of both components can be different from the receptive field in response to individual pheromone components. Receptive fields of different PNs in the MGC of one animal reflect the heterogeneity in this population of PNs, whereas in other cases PNs also showed similar receptive fields, possibly based on similar dendritic arborizations in the MGC. Different receptive field properties of PNs potentially function during different phases of upwind flight and search behavior of male moths.

Supported by NIH grant AI23253 to J.G.H.

Local Interneurons Define Functionally Distinct Layers in the Olfactory Glomeruli of the Spiny Lobster

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Glomeruli in the olfactory lobe (OL) of the spiny lobster are columnar, not spherical, with three morphologically distinct regions: a peripheral cap, an underlying subcap and a proximal base. Receptor neurons innervate all three regions, but branch most heavily in the cap and subcap (Schmidt and Ache, J. Comp. Neurol.318:291,1992). The projection neurons branch most heavily in the base region (Wachowiak and Ache, in press). Whole-cell recording with biocytin-filled electrodes reveals that local interneurons (LNs) confine their branching to these morphological regions, suggesting that the regions represent functionally distinct zones within the glomerulus. LNs can be identified by their pattern of branching within the zones: Type I - branches and terminates only in the cap, Type II - branches and terminates in both the cap and subcap, Type III - branches and terminates in the base,subcap and cap; and, Type IV - branches in the base but terminates only in the subcap. Two additional types, which also branch in a related neuropil, show the same type of branching in the OL: Type V - branches and terminates in the base, subcap and cap, and Type VI - branches and terminates in the subcap and/or upper base. LNs that branch primarily in the cap and subcap (Types I,II,and VI) innervate >50% of the approximately 1100 glomeruli in the OL, in contrast to the LNs that branch primarily in the base (Types III and V), which innervate from 2-10 glomeruli. The LNs appear to define at least two, possibly sequential, layers of processing in the OL. Widely branching LNs distal in the OL may serve to focus afferent input by enhancing contrast among glomeruli, while less widely branching LNs proximal in the OL may serve the processing of this focussed input by the output neurons. This organizational scheme would be reminiscent of processing in the vertebrate olfactory bulb and possibly indicate the fundamental importance of this strategy for odor processing at the first synaptic level of the olfactory pathway.

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Dual inhibitory inputs to olfactory projection neurons in the olfactory lobe of the lobster

M. WACHOWIAK and B.W. ACHE (Whitney Laboratory and Dept. Neuroscience, Univ. Florida, St. Augustine, FL, 32086).

Inhibitory inputs to olfactory projection neurons in the olfactory lobe (OL) of the lobster were investigated using pharmacological probes for GABA and histamine, two presumptive inhibitory transmitters localized immunohistochemically to the lobster OL (Orona et al., J .Comp. Neurol. 294:633, 1990). Drugs were perfused into an isolated brain preparation and their effect on OL projection neurons was measured via whole-cell current-clamp recording from the cell somata. As reported last year, OL projection neurons have a high input resistance (approx. 1 GΩ) and respond to high-intensity electrical stimulation of the antennular (olfactory) nerve with a brief depolarization and spike burst followed by a 1-2 sec hyperpolarizing IPSP; weak stimulation elicits only a hyperpolarizing IPSP with no spiking. Histamine reversibly increased the latency to spiking and increased the threshold for eliciting spiking responses. Cimetidine, a histamine H2-receptor antagonist, reversibly decreased the threshold for eliciting spiking without affecting spike latency and, in some preparations, caused the IPSP to be replaced by a sustained depolarization lasting up to 8 seconds. These results suggest that the hyperpolarizing IPSP seen in OL projection neurons is at least partly mediated by histamine, and that histamine may also act to inhibit neurons presynaptic to projection neurons. GABA also reversibly increased the threshold for eliciting spiking, but additionally caused up to a ten-fold decrease in the somatic input resistance of projection neurons, greatly attenuating spike amplitude and eliminating subthreshold potential changes as measured at the soma. The effect of GABA on response threshold implicates this transmitter in inhibitory glomerular processing. The effect on input resistance suggests additional, extra-glomerular GABA-ergic input to OL projection neurons, since the sites of glomerular input are electrotonically distant from the recording site at the soma. These results confirm the suspected role of GABA and histamine as inhibitory transmitters in the OL, and suggest that at least two functionally distinct inhibitory pathways shape the output of glomerular-based processing of olfactory information in the lobster.

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Statistical Characterization and Dynamical Analysis of Local Temporal Structure of Spike Trains.

JON C. WEIL and STEPHEN P. FRACEK, JR.
(University of North Texas and Center for Network Neuroscience)

We are interested in determining if the extracellularly recorded activity of cultured olfactory bulb cells is modified when the bulb cells are co-cultured with olfactory epithelia and exposed to odorants. We are developing data analysis techniques that describe temporal changes in the interspike intervals. Bicuculline (1 to 100 μM) causes reliable changes in the spiking activity. We now report on a technique that describes the statistical serial dependence of interspike interval data. Single unit interspike interval sequences are ranked while preserving their temporal order. We form n step Markov Chains (currently $n \leq 6$) from these data. Plots of conditional and joint probability of aggregate states provide qualitative statistical information about the local temporal structure of the spike train. An extension of the Joint Interval Histogram, these plots express all occurring sequences (up to n length) in the context of all possible sequences. This method may show nonlinear correlation, whereas autocorrelation shows only linear correlations. Examination of the plots as n varies yields information about the dynamical evolution of the spike train statistics. Furthermore, specific hypotheses concerning temporal patterns can be identified using templates or statistical tests.

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Evidence For Glutamate As The Olfactory Nerve Neurotransmitter. D.A. BERKOWICZ *, P.Q. TROMBLEY and G.M. SHEPHERD. (*Interdepartmental Neuroscience Program and Section of Neurobiology, Yale University School of Medicine, New Haven, CT 6510.)

In the vertebrate olfactory system odor stimuli are transduced by olfactory receptor neurons (ORNs) into impulses which are transmitted via the olfactory nerve to the primary output neurons of the olfactory bulb, the mitral/tufted (M/T) cells. Synaptic transmission from ORN axon terminals to M/T cell dendrites is known to be excitatory. However, the neurotransmitter used by ORNs is still unidentified. The dipeptide carnosine has been the most frequently proposed candidate, but thus far there is no evidence for a direct action of carnosine on olfactory bulb neurons. The possibility that glutamate mediates transmission between ORNs and M/T cells is supported by the recent immunocytochemical study of Sassoe-Pognetto et al. (1993) which demonstrated the presence of glutamate in the ORN axon terminals. In order to test the hypothesis that glutamate is the ORN neurotransmitter we developed a hemisection preparation of the turtle olfactory bulb and used whole cell current clamp recording techniques to characterize the properties of synaptic transmission from ORNs to M/T cells. A synchronous impulse volley evoked by a nerve shock resulted in a rapid and sustained depolarization in M/T cells. This depolarization was closely mimicked by the application of 1mM glutamate. The response to a nerve volley consisted of two components. The first, a rapid depolarization of short duration, was sensitive to the AMPA receptor antagonist 6,7-Dinitroquinoxaline-2,3-dione (DNQX); the second, a depolarization of slower onset but longer duration, was sensitive to the NMDA antagonist 2-amino-5 phosphonopentanoic acid (AP5). When DNQX and AP5 were both present the postsynaptic response was completely abolished. These data strongly support the notion that glutamate is the neurotransmitter at this synapse.

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Excitatory Amino Acid Receptor Antagonism Blocks Sensory Evoked Excitation of Rat Mitral Cells in Vitro

M. ENNIS, M. JIANG, L.A. ZIMMER, & M.T. SHIPLEY
(University of Cincinnati College of Medicine)

Mitral cells of the main olfactory bulb (MOB) receive direct excitatory synaptic inputs from the terminals of primary olfactory axons. The identity of the excitatory transmitter(s) in the olfactory nerve is the subject of much interest. However, soluble molecules known to be present in olfactory nerve, such as carnosine, do not have an identified postsynaptic action in the bulb. Excitatory amino acids (EAAs) are strong candidates for olfactory nerve->mitral cell transmission as they mediate fast excitatory synaptic transmission in other systems and markers for the major EAA receptor subtypes are present in the glomerular layer. Recently, Berkowicz et al. (1993) reported that olfactory nerve-evoked excitation of mitral cells was blocked by EAA receptor antagonists in turtle bulb slices.

Here, we have examined the role of EAAs in olfactory nerve->mitral cell transmission in a mammalian *in vitro* bulb slice preparation. Olfactory bulbs were rapidly removed from chloral hydrate-anesthetized rats (50 - 120 g) and 400 μm-thick horizontal vibratome sections were harvested. Slices were submerged in a recording chamber and a bipolar stimulation electrode was placed on the olfactory nerve layer (ONL). Extracellular recordings were obtained from mitral cells with dye-filled glass pipettes.

Single pulse (0.5 Hz), low intensity (25-500 μA) ONL stimulation evoked robust excitatory responses in most mitral cells tested, with onset latencies of 5-10 msec. Typically, mitral cells responded to ONL stimulation with 1 or 2 driven spikes followed by a 20-100 msec epoch of inhibition. Less frequently, single ONL shocks elicited bursts of spikes from mitral cells lasting up to 1 sec in duration.

Bath application of the broad spectrum EAA antagonist kynurenic acid (KYN, 5 mM) rapidly and completely blocked ONL-evoked excitation of all mitral cells tested (n = 7). KYN blocked both single spike and burst responses to ONL stimulation. In all 4 cells held for a sufficient period of time, ONL-evoked excitation of mitral discharge began to recover approximately 5-15 min after termination of KYN perfusion; full recovery was observed after 20-30 min. KYN had no consistent effect on the spontaneous discharge rate of mitral cells.

The present results suggest that glutamate or aspartate may be an *endogenous* transmitter mediating olfactory nerve->mitral cell excitatory synaptic transmission. As KYN is a broad spectrum EAA antagonist, our results do not allow identification of the specific receptor subtype(s) involved. Additional studies using selective EAA receptor subtype antagonists are in progress to address this issue.

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Generators of the P90 Component of the Olfactory Evoked Potential in Rats TU-UYEN NGUYEN and W. JAMES EVANS
(University of California, Irvine)

We have previously reported that phase reversal of the P90 component of the olfactory evoked potential in rats to amyl acetate occurs within 1 mm of the bulb surface. The current experiment attempts to verify histologically the site of this phase reversal. Evoked potentials were recorded from the olfactory bulb in anesthetized rats in response to odorant stimuli presented through a nasal cannula. A constant flow olfactometer delivered stimuli consisting of 10% amyl acetate in diethyl phthalate at a flow rate of 1 L/min, stimulus duration of 20 ms, and an interstimulus interval of 8 s. Craniotomy was performed to expose the olfactory bulb, and a single electrode was positioned deep within the olfactory bulb in the granule cell layer. Subcutaneous electrodes were also placed in the scalp over the nose. Evoked potentials were recorded as the bulb electrode was moved up through the more superficial layers of the bulb. At the level of phase reversal, an electrolytic lesion was produced. Histological sections of the bulb stained with Cresyl Violet localized the lesion, and hence, the site of phase reversal, to the mitral cell layer. Based on current source density analysis of similar olfactory bulb potentials by Martinez and Freeman (Brain Res., 1984) using electrical stimulation of primary olfactory nerve, our current results suggest that the P90 component results largely from depolarization of granule cells.

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Voltage Dependence of Long Duration Olfactory Nerve Evoked Synaptic Potentials in Rat Olfactory Bulb Mitral Cells.
W. T. NICKELL (U. of Cincinnati), M. T. SHIPLEY (U. of Maryland) and M. M. BEHBEHANI (U. of Cincinnati).

Electrical stimulation of the olfactory nerve (ON) produces a long duration (>500 msec) excitation of mitral cells in turtle olfactory bulb (OB) following blockade of inhibition. We observed a similar long duration excitation in rat olfactory bulb slices without blockade of inhibition, presumably because activity of the mitral/granule inhibitory system is much reduced in the slice. Because of the probable importance of this long duration excitation to theories of olfactory bulb function, we investigated the effect of membrane potential on the amplitude and duration of ON evoked potentials in rat OB mitral cells. Rat OBs were cut in approximately horizontal 400 μm slices. Whole cell patch techniques were used to record the synaptic response to a shock applied to the olfactory nerve layer. To block sodium spikes in the patched mitral cell while maintaining function of the nerve, patch pipettes were filled with 0.5 to 1 mM QX 314, an internally acting blocker of fast sodium channels. After impalement of a mitral cell with a patch pipette filled with QX 314, sodium spikes were usually completely blocked within 10 minutes. Hyperpolarization of the soma sharply decreased the duration of the EPSP. Amplitude of the EPSP did not increase linearly with membrane potential. Together, these observations suggest that the underlying conductance change is voltage dependent. When the soma was depolarized, large potassium conductances prevented determination of the reversal potential of the synaptic response. After block of potassium currents with extracellular TEA or 4-AP, depolarization resulted in slow, large amplitude inward currents. These slow currents may partially account for the long duration of the olfactory nerve evoked EPSP.

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CSERPs to hedonically pleasant and unpleasant stimuli.
TYLER S. LORIG, DOUGLAS C. MATIA, JAMES M. TURNER, (Washington and Lee University) , STEPHEN WARRENBURG, (IFF), and GEORGE PRETI (Monell Chemical Senses Center and University of Pennsylvania).

Kobal and colleages have found that the hedonic quality of an odor may be coded very early in odor perception suggesting that hedonics may not rely exclusively upon cognition. In an effort to replicate and extend these findnings to other odorants, an experiment involving fifteen subjects was conducted using phenethyl alcohol and 3-methyl-2-hexenoic acid (3M2H). Concentrations were matched for intensity and administered via a computer-operated olfactometer in a warmed and humidified constant airstream. Administrations were 0.25 sec and were delivered independently to each nostril. ERP data within a 0.0 - 20 Hz band pass were collected from 30 electrode locations using an averaged reference. Peaks of the N1 - P2 component, averaged over the electrodes of the left and right hemispheres, are presented below. ANOVA indicated a significant interaction of odorant, nostril of administration, and hemisphere such that PEA produced its largest response in the right hemisphere during left nostril administration. Odors also differed in their latency with both N1 and P2 occuring earlier for 3M2H Acid.

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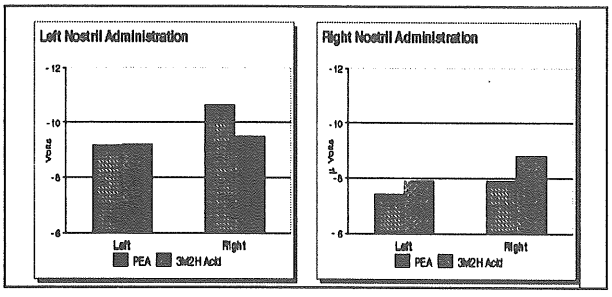

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EEG Registration of Unconscious Odor Concentrations of Isoamyl Acetate: A Double Blind Experiment
GARY E. SCHWARTZ, JOHN P. KLINE, ZIYA V. DIKMAN, and MERCEDES FERNANDEZ (University of Arizona)

Nineteen channels of EEG were recorded from 30 college students while they sniffed pairs of bottles. One bottle of a pair contained isoamyl acetate (IAA) in silicone. The other bottle of this pair contained silicone (the control). Subjects were prescreened for IAA detection. EEG was collected during two two-second sniff periods per bottle for 8 trials per odor at two concentrations, one suprathreshold and one subthreshold. Experimenters and subjects were blind to which bottles contained the subthreshold concentration. The order of odors, concentrations, experimental and control bottles, and hand, was counterbalanced within subjects. After each trial, subjects indicated which bottle contained the odor (detection), rated their confidence of detection and the odor's intensity. EEG was amplified using the NeuroSearch-24. EEG was sampled at 256 Hz, spectral analyses were performed in 2 Hz bands on 8 sec of EEG per trial, and odor minus control relative magnitude scores were displayed in topographic maps. Correct odor detections for the suprathreshold concentration was 99% and for the subthreshold concentration was 51% (chance=50%). When subjects sniffed subthreshold IAA, significant decreases in theta and alpha were observed in central and posterior regions. 4-6 Hz theta showed the strongest effects for the first sniff, 6-8 Hz theta showed strong effects for both sniffs, 8-10 Hz alpha showed stronger effects for the second sniff, and 10-12 Hz alpha showed the strongest effects for the second sniff. These data demonstrate that humans can register odors at subthreshold levels. The data suggest that there is a relationship between unconscious information processing and EEG frequency.

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EEG registration of Androstenone Odor Response in Androstenone Anosmic Subjects
GARY E. SCHWARTZ, ZIYA V. DIKMAN, JOHN P. KLINE, MERCEDES FERNANDEZ, and ERNEST H. POLAK (University of Arizona)

Nineteen channels of EEG were recorded from 86 college students while they smelled pairs of bottles. One bottle per pair contained androstenone (AND) (5-alpha-androst-16-en-3- one) dissolved in silicone. The other bottle contained silicone (the control). EEG was collected during two two-second sniff periods per bottle for 8 trials at two concentrations, one suprathreshold and one subthreshold (subthreshold AND determined by AND osmic subjects). The order of odors, concentrations, experimental and control bottles, and hand, was counterbalanced within subjects. After each trial, subjects indicated which bottle contained the odor (detection), rated their confidence of detection and the odor's intensity. EEG was amplified using the NeuroSearch-24. EEG was sampled at 256 Hz, spectral analyses were performed in 2 Hz bands, and odor minus control relative magnitude scores were displayed in topographic maps. Correct odor detections for the suprathreshold concentration of AND was 95% for osmics (n=59) and 42% for anosmics (n=27) (chance=50%) EEG registration of AND was dramatically different for alpha versus theta bands in osmics versus anosmics. For osmic subjects, alpha decreases occurred for both sniffs. For anosmic subjects, alpha decreases were observed primarily in posterior regions in sniff 2. However, both osmic and anosmic showed theta decreases in sniff 1, and anosmic subjects showed theta increases in sniff 2. These data are consistent with the hypothesis that anosmia for androstenone involves central mechanisms, that AND anosmics may be rapid CNS adaptors, and that there is a relationship between unconscious information processing and EEG frequency.

Antisense Oligonucleotides to Perturb Calmodulin Function in Chemoresponse.

JUNJI YANO, FRANK HECHT, and JUDITH VAN HOUTEN (University of Vermont, Dept. Zoology, Burlington, VT 05405).

In *Paramecium* chemoresponse, the receptors for some stimuli appear to couple to a calcium pump rather than to a channel to generate a hyperpolarizing conductance. The pump is likely to be the plasma membrane Ca-ATPase that is involved in homeostasis and it would be difficult to select even conditional mutations in the gene for this protein. Since the pump is calmodulin regulated, it should be possible to alter pump activity indirectly by altering calmodulin. We down regulate calmodulin as first shown by Hinrichsen et al. (PNAS 89: 8601-5, 1992) using antisense oligonucleotides, complementary to the 5' end of the calmodulin mRNA. The oligonucleotides have cholesterol at the 3' end to slow degradation upon electroporation into the cell. The efficiency of transformation ranges from 10-30% as judged by the cells' swimming behavior. When calmodulin is defective (as in some *Paramecium* mutants), either a calcium activated Na or K conductance is defective. The loss of the calcium activated conductances shows up as altered swimming behavior. A turn in swimming path is triggered by a calcium action potential. The calcium activated Na conductance can prolongs the plateau of the action potential and prolong the backward swimming phase of the turn. The loss of the calcium activated K conductance has the opposite effect. The Na conductance is more sensitive to calmodulin levels and, when antisense oligonucleotides for calmodulin are electroporated, this conductance drops out first and we monitor the transformation by loss of backward swimming in depolarizing stimuli. Preliminary experiments show that cells with this altered behavior show reduced calcium activated K conductance, thus for the first time indirectly confirming that calmodulin function is reduced. More interestingly, these cells also appear to fail to respond to some attractant chemical stimuli. This work is supported by NIH and the VCCC.

Intracellular pH During Chemoreception in *Paramecium*.

DAVID DAVIS AND JUDITH VAN HOUTEN (Dept. of Zoology, University of Vermont).

Most attractant stimuli hyperpolarize *Paramecium* cells and cause the characteristic changes in swimming behavior that lead to attraction by a kinesis mechanism. The hyperpolarizing conductances seem to be due to the activation of a calcium hoemostasis pump for one class of stimuli and involves cyclic AMP as a second messenger for a second class of stimuli. However, NH_4Cl is a third kind of attractant stimulus that does not necessarily require a receptor to initiate the signal transduction pathway and appears to involve neither cyclic nucleotides nor the calcium pump. To investigate a possible mechanism of signal transduciton initiated by this stimulus, we measured intracellular pH with ion-sensitive fluorescent dyes and examined the motility changes of cells in control and stimulus solutions. We examined both wild type cells and mutants that were previously selected for their inability to show attraction to NH_4Cl.

NH_4Cl characteristically, rapidly alkalinizes the intracellular pH when cells enter this solution and perhaps this alkalinization is part of the signal transduction mechanism that results in hyperpolarization. Surprisingly, the cells acidify while in NH_4Cl over a period of minutes. Two mutants analyzed to date do not show this same pattern of changes in intracellular pH. Another attractant stimulus, acetate, slowly acidifies the cells, as would be expected. However, these stimuli do not initially alkalinize and utilize a different transduction pathway.

The wild type cells swim smoothly and relatively fast in NH_4Cl, as expected for relatively hyperpolarized cells, and show attraction to NH_4Cl in T-maze assays. The mutants cells are being examined by motion analysis but have been shown to be defective in attraction to NH_4Cl in T-mazes.

This work is supported by NIH and the VCCC.

Effect of modulators of the phosphotidyl inositol system and the arachidonic acid system on sweet electrophysiological taste responses in gerbil. SUSAN S. SCHIFFMAN (Duke University), MARK S. SUGGS (Duke University) and MICHAEL L. LOSEE (The Nutrasweet Co.).

The adenylate cyclase cascade is a second messenger system that has previously been shown to be involved in both sweet and bitter taste transduction. The purpose of the present study was to determine if two other second messenger systems, the phosphotidyl inositol system and the arachidonic acid system, also play a role in taste transduction. Modulators of the phosphotidyl inositol system and the arachidonic acid system were applied to the gerbil tongue to determine if they alter sweet taste responses. Integrated chorda tympani (CT) recordings were made before and after a four minute application of several types of modulators of the phosphotidyl inositol system and the arachidonic acid system to determine their effect on sweet taste. The sweet compounds tested were: sucrose (30 mM and 100 mM), glucose (300 mM), fructose (300 mM), maltitol (150 mM and 300 mM), mannitol (300 mM and 500 mM), sodium saccharin (10 mM), D-tryptophan (6.5 mM), dulcin (0.88 mM, and 3.5 mM) and stevioside (0.55 mM and 1.1 mM). Bitter, salty, sour, and glutamate tastants were also tested as controls. These compounds were: NaCl (30 mM and 100 mM), KCl (300 mM and 500 mM), NH_4Cl (100 mM), monosodium glutamate-MSG (50 mM and 100 mM), HCl (5 mM and 10 mM), quinine HCl (30 mM), $MgCl_2$ (30 mM and 100 mM), $CaCl_2$ (30 mM, 100 mM, and 300 mM), and urea (2 M). The modulators of the phosphotidyl inositol system were: 1-oleoyl-2-acetyl glycerol (OAG) and dioctanoyl glycerol (DiC8), two membrane permeable analogues of DAG; thapsigargin, which releases Ca^{++} from intracellular stores; ionomycin, a calcium ionophore; lanthanum chloride, an inorganic channel blocker; and nifedipine, a dihydropyridine calcium channel blocker. Modulators of the arachidonic acid system were: quinacrine diHCl, a phospholipase A_2 antagonist, melittin, a 26 amino acid amphipathic polypeptide toxin that is a phospholipase A_2 agonist, and indomethacin, which decreases the release of prostaglandins by inhibiting the enzyme cyclo-oxygenase. Arachidonic acid was also tested for its effect on taste. The main findings of this study were: OAG (125 μM) produced a decrement in the responses to: 30 mM $MgCl_2$ (29%) and 2 M Urea (40%), while enhancements in responses were found for: 300 mM fructose (21%), 300 mM maltitol (38%) and 0.55 mM stevioside (18%). DiC8 (100 μM) blocked the responses of several bitter compounds while enhancing the taste response to some sweeteners. In addition, lanthanum chloride (0.01 mM, 0.10 mM, 1 mM and 10 mM) had significant effects on most of the stimuli tested, with overall blockages of (15%, 33%, 40% and 72% respectively). Melittin (70 μM) showed an overall reduction in all taste responses of 50%.

Effect of environmental pollutants on taste in gerbil. SUSAN S. SCHIFFMAN (Duke University), MARK S. SUGGS (Duke University), M. B. ABOU DONIA (Duke University), H. T. NAGLE (N.C. State University), and R. P. ERICKSON (Duke University).

The chemical senses of taste and smell play a crucial role in food selection and intake. Damage to the chemical senses can impair food intake, nutritional status, and survival. The purpose of this study was to determine the effects of eleven environmental pollutants (nine insecticides and two herbicides) on taste responses in gerbil. Integrated chorda tympani (CT) recordings were made in gerbils to a range of tastants before and after a four minute application of eleven environmental pollutants. The taste stimuli tested were: NaCl (100 mM), $CaCl_2$ (300 mM), $MgCl_2$ (100 mM), HCl (10 mM), KCl (500 mM), monosodium glutamate-MSG (50 mM), sucrose (100 mM), fructose (300 mM), sodium saccharin (10 mM), quinine HCl (30 mM), and urea (2 M). The nine insecticides tested included organophosphorous, carbamate, and pyrethroid insecticides. The seven organophosphorous insecticides tested were: acephate, carbofuran, chlorpyrifos, chlorpyrifos oxon, demeton, malathion, and methamidophos. The carbamate insecticide carbaryl and the pyrethroid insecticide fenvalerate (sanmarton) were also tested. The two herbicides tested were paraquat and glyphosate. Dose response curves for each of the two herbicides were also determined. Each of the eleven insecticides and herbicides had a significant effect on some of the taste stimuli tested. Application of 10 mM methamidophos exhibited the greatest amount of suppression on the eleven taste solutions. Each taste stimulus was significantly suppressed by at least 19.6% with the exception of 10 mM sodium saccharin and 2 M urea. Herbicides paraquat and glyphosate also reduced responses to several tastants. These data indicate that environmental pollutants can modify taste responses in gerbil.

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Taste-guided Unconditioned Licking to Suprathreshold Sodium Chloride Solutions is Unaffected by Selective Lingual Denervation. RAY CAUTHON, MIRCEA GARCEA, and ALAN C. SPECTOR (Dept. of Psychology, Univ. of Florida).

Electrophysiological data suggest that the chorda tympani nerve (CTn) makes a unique contribution to the peripheral signal representing sodium chloride (NaCl). In support of this hypothesis, bilateral section of the CTn (CTx) in rats, raises the NaCl detection threshold by about two orders of magnitude. We evaluated the effect of CTx on unconditioned suprathreshold responsiveness to NaCl. Water-deprived rats were presented with different NaCl solutions (.03 - 1.0 M) and distilled water in a specially designed gustometer. The tastants were delivered in randomized blocks of 10 s trials, and the number of licks to each stimulus was recorded during three 40 min sessions both before and after surgery. All surgical manipulations were confirmed histologically. Presurgical and postsurgical concentration-response functions were compared in separate 2-way ANOVAs to determine the effect of each of the surgical manipulations on NaCl responsiveness. All groups reliably decreased licking as a function of NaCl concentration both before and after surgery. Responsiveness to NaCl was relatively uncompromised by bilateral transection of the CTn (n=8) or glossopharyngeal nerve (GLn, n=6). In fact, responsiveness to 1.0 M NaCl was enhanced by CTx as indicated by the decrease in licks to this stimulus after surgery. More surprisingly, in rats receiving combined bilateral transection of the CTn and GLn (n=6), which removes about 80% of the taste buds, NaCl responsiveness was also relatively unaffected. In contrast, rats that had their sublingual and submaxillary salivary glands extirpated (n=6) were significantly less responsive to the mid-range concentrations of NaCl, licking these taste stimuli more under water-deprived conditions after surgery as compared with before surgery. These results indicate that the sublingual and submaxillary salivary glands are necessary for maintaining normal unconditioned responsiveness to NaCl under conditions of water deprivation. These findings also reveal that such responsiveness can be apparently maintained by gustatory nerves other than the CTn and GLn.

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Differences in the Spectral Tuning Properties of Front and Rear Legs of lobsters: Functional Adaptation?. RAINER VOIGT, KEITH M. BAYHA and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

The fourth pair of walking legs in the American lobster, *Homarus americanus*, differs in morphology and behavioral function from the two first pairs. Apart from their obvious use in walking, the first two pairs of walking legs are chelated and serve in feeding behavior, i.e., locating, grasping and transporting of food to the maxillipeds and grooming of the anterior body. The fourth pair of walking legs is used for walking and grooming of the posterior body. In the egg-bearing female, this includes prominently the grooming of the egg mass. Such behavioral differences may correlate with physiological differences in chemoreceptor function between the two leg pairs. We therefore determined the spectral sensitivity of chemoreceptor cells in the first and fourth walking legs focusing on chemicals present in food odor and products of bacterial degradation. Because of their egg-cleaning behavior, we used female lobsters for this initial study. We assessed the tuning properties of chemoreceptor cells by measuring responses to 18 single chemical stimuli (each at 10^{-3} M); standard electro-physiological methods using suction electrodes were applied to record extracellular action potentials. The first and fourth pairs of legs had different effective stimuli. Hind leg chemoreceptors responded best to hydroxy-L-proline, followed by glycine, methionine, glutamine, betaine, and leucine. Front leg chemoreceptors, on the other hand, responded best to ammonium chloride, followed by glutamate, lysine, leucine, betaine and taurine. In addition, chemoreceptor cells in the hind legs were more broadly tuned than those in the front legs. Our results indicate that the front and hind legs have different spectral sensitivities which might be necessary for different behavioral functions.

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Protein Tyrosine Kinases and Helix-Loop Helix Proteins in Taste Tissue.
SUSAN MCLAUGHLIN and ROBERT F. MARGOLSKEE (Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110).

Little is known about the molecular mechanisms which control taste bud development and taste cell differentiation. Many proteins which regulate differentiation and development contain conserved functional motifs, including basic helix-loop-helix regions, Pax domains, homeobox regions, and tyrosine kinase domains. We used the PCR to screen a taste-enriched cDNA library for the presence of two of these gene families: basic helix-loop-helix proteins, and protein tyrosine kinases. Twenty-four protein tyrosine kinases and three helix-loop-helix proteins were isolated from the taste enriched cDNA library. RNase protection, *in situ* hybridization, and immunohistochemistry were used to examine the relative levels of expression of the isolates in taste tissue compared with non-taste lingual epithelium. Ten protein tyrosine kinases and one basic helix-loop-helix protein had elevated levels of expression in taste tissue, suggesting they may regulate taste cell functions.

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G Protein Phosphodiesterase Interactions in Sensory Transduction: Effector Interaction Peptides from the Transducins and Gustducin Activate Phosphodiesterases.
NANCY SPICKOFSKY, WALEED DANHO and ROBERT F. MARGOLSKEE (Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110).

Gustducin (McLaughlin *et al.*, Nature **357**:563-569, 1992) is a taste cell specific G protein whose α subunit is 80% identical to those of rod and cone transducin (the photoreceptor G proteins). We recently found that rod transducin is also expressed in taste cells (McLaughlin *et al.*, Ciba Foundation Symp. **172**:196-200, 1993). In vertebrate rod cells, rod transducin couples rhodopsin to cGMP phosphodiesterase: activated rod transducin α interacts with the γ subunit of phosphodiesterase to disinhibit the catalytic subunits of phosphodiesterase. Rarick *et al.* (Science **256**:1031-1033, 1992) have recently identified a 22-amino acid long peptide from rod transducin's effector interaction domain that activates phosphodiesterase. Within this region there are only a few sequence differences between rod transducin and its most closely related homologues: cone transducin and gustducin. Although the rod transducin peptide activated rod cGMP phosphodiesterase as well as did GTPγS activated rod transducin α, effector interaction peptides from cone transducin, gustducin, and $\alpha_{i\text{-}1,2,3}$ were all incapable of activating rod phosphodiesterase. Using substituted peptides differing from the rod peptide at 1 or 2 positions we have identified those residues that are particularly important to the rod transducin-rod cGMP phosphodiesterase interaction. These results indicate that between 2 and 4 differences between rod transducin α and closely related G protein α subunits specify the ability or inability to activate rod cGMP phosphodiesterase. We are carrying out analogous studies with cone cell and taste cell phosphodiesterases to determine if similar G protein-phosphodiesterase interactions occur in gustducin and transducin containing taste cells and in cone cells of the retina.

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A Metabotropic Glutamate Receptor is Expressed in Rat Taste Buds
CHAUDHARI, N., H. YANG, C. LAMP, (Department of Physiology, Colorado State Univ.) and S. ROPER (Department of Anatomy & Neurobiology, Colorado State Univ.; Rocky Mountain Taste & Smell Center, Univ. Colorado Health Sciences Center)

Glutamate is an important sapid stimulus thought to evoke a distinctive taste, *umami*. Glutamate may also be a neurotransmitter in taste buds (Lu & Roper, 1993). We recently reported that a number of different glutamate receptors (GluRs), originally cloned from the brain, are found in lingual epithelium of rats (Chaudhari *et al.*, Proc. ISOT 1993). Using reverse transcriptase-PCR and degenerate PCR primers, we have found that a single metabotropic (i.e. G-protein-coupled) receptor type, mGluR4, is associated with vallate papillae. Lingual epithelium containing few or no taste buds did not yield an amplification product corresponding to mGluR4. We have now localized mGluR4 to vallate taste buds, using *in situ* hybridization. ^{33}P-labeled RNA probes specific for mGluR4, in sense and antisense orientation, were synthesized at high specific activity. These probes were used to hybridize mRNA in frozen sections of fixed rat tongue. We followed the protocol of McLaughlin and Margolskee (1993), with modifications for detecting rare mRNAs. With autoradiographic exposures of 4-8 weeks, clusters of silver grains were detected over vallate taste buds. Background grain density over connective tissue, lingual glands and non-taste epithelium was considerably lower than over taste buds. Sections hybridized with sense probes showed a similarly low background density of grains. Interestingly, we found that only 10-30% of the taste buds within any vallate papilla contained mRNA for mGluR4. We believe this is the first report of a membrane receptor that has been selectively localized to mammalian taste buds. Whether mGluR4 represents a receptor for taste molecules or for neurotransmitter remains to be ascertained.

We acknowledge the support of the Umami Manufacturers Association of Japan, Kraft General Foods, and National Institutes of Health (DC00374).

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Using CTA to Characterize Glutamate Receptors Underlying MSG Taste
CARTFORD, C., T. THAN, E. DELAY (Dept of Psychology, Regis Univ., Denver), and S. ROPER (Dept of Anatomy & Neurobiol, Colo St Univ; Rocky Mt. Taste & Smell Center, Univ. Colo Health Science Cntr)

MSG is an important taste enhancer and is thought to evoke a distinctive taste, *umami*. Transduction mechanisms underlying MSG taste are not known. One hypothesis is that glutamate receptors, similar to those that mediate synaptic transmission in the brain, are present in the apical membrane of taste cells. There are 4 classes of brain glutamate receptors, differing in their molecular structure and pharmacological profiles. These are: AMPA; kainate; NMDA; and metabotropic receptors. Each binds glutamate as well as the specific ligand which differentiates the class. That is, AMPA, kainic acid (KA) and n-methyl d-aspartic acid (NMDA) activate AMPA, kainate and NMDA receptors, respectively. L-AP4 selectively activates metabotropic glutamate receptors. We are investigating which receptor(s) underlies MSG taste by using CTA to determine whether the taste of MSG generalizes to the taste of any of these ligands. The first task was to establish a concentration range for each of the ligands that was effective in the CTA paradigm. Male albino rats were presented with 12 trials, each 10 sec in duration, of one of the following: MSG, AMPA, KA, NMDA or L-AP4. All stimuli were presented at pH 6~7. Each presentation was alternated with distilled water. To avoid the potential confounding influence of Na^+ taste in MSG, 30 μM amiloride was present in all solutions, including distilled water. The rats were then injected with 1 M LiCl (@127 mg/kg) or saline solution (controls) and tested 2 days later for aversion to the conditioned stimulus. To control for non-selective avoidance of all taste stimuli on the test day, we included presentations of 100 mM sucrose, 25 mM KCl and distilled water. Rats readily imbibed all solutions and reliable conditioned aversion was established with the following concentrations: MSG (100 mM); AMPA (10 mM); KA (25 mM); NMDA (50 mM); L-AP4 (10 mM). Our data to date indicate that it is possible to establish conditioned aversion to each of these substances and that the aversion is dose-dependent. We confirmed that aversion to MSG generalizes to sucrose when amiloride is present (Yamamoto, *et al* ., 1991). We are presently testing whether aversion to MSG generalizes to any of the specific glutamate receptor ligands.

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Ultrastructure of Electrophysiologically Distinct Taste Receptor Cells in *Necturus* Lingual Slices.
STEWARD, D.E., A. BIGIANI, and S.D. ROPER (Dept of Anatomy & Neurobiology, Colorado State Univ.; Rocky Mt. Taste & Smell Center, Univ. Colorado Health Sciences Center)

In taste buds, light and electron microscopic studies have revealed the existence of different morphological types of receptor cells (*e.g.*, dark and light cells). However, little is known about the physiological properties of these cell types and how the different cells participate in taste transduction. Recent electrophysiological findings have shown that there is a functional diversity among *Necturus* taste receptor cells regarding their membrane ionic conductances (Bigiani & Roper, 1993). We have approached the problem of correlating the electrophysiology and morphology of taste cell types by combining whole-cell patch clamp recordings with light microscopic (LM) and electron microscopic (EM) observations in a slice preparation of *Necturus* lingual epithelium. To identify taste receptor cells at the LM and EM levels, Lucifer yellow (0.2%) and biocytin (0.5%) were included in the recording patch pipette solution. After electrophysiological recordings, the tissue was photographed with fluorescence microscopy. The tissue was then reacted with diaminobenzadine to visualize biocytin, photographed again, and then processed for EM. In this way, we could correlate light micrographs with subsequent electron micrographs and reliably relocate labelled taste cells. Two groups of taste receptor cells could be distinguished on the basis of their whole-cell currents: (a) cells with voltage-gated Na^+ and K^+ currents (15 out of 21 cells); (b) cells with voltage-gated K^+ currents only (6 out of 21 cells). To date, we have correlated the electrophysiology and electron microscopy on 3 of these taste cells. Cells that had voltage-gated Na^+ and K^+ currents (N=2) possessed numerous granular packets in the apical process, a consistent diagnostic feature for dark cells. In contrast, the cell with only voltage-dependent K^+ currents lacked granular packets and possessed abundant smooth endoplasmic reticulum, indicating that it was a light cell. The data indicate that electrophysiological properties might be readily correlated with specific morphological types. These preliminary results also suggest that dark cells can generate action potentials whereas light cells are electrically inexcitable. We are increasing our sample size to test whether these preliminary observations are maintained and thus if this generalization can be considered a basic feature of the biology of taste buds.

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Comparative Features Among Fungiform Papillae of Rabbits, Humans and Monkeys.
INGLIS MILLER, JR. (Dept. of Neurobiology & Anatomy, Wake Forest University, Winston-Salem, NC 27103)

Taste-mediated behaviors are related to taste bud prevalence in several species of mammals. An effort has been initiated to identify anatomical attributes which associate with the relative abundance of taste buds in fungiform papillae. Videomicroscopy is used to characterize papillae and quantify taste pores of living specimens and fixed tongues of humans (N = 39), rabbits (N = 27) and Cynomolgus monkeys (N = 11). Among all 3 species, taste pores on fungiform papillae are restricted in location to the apical surfaces of papillae. The epithelia on these apical surfaces of "fungiform" papillae do not stain with methylene blue except in the region of taste pores. Methylene blue stains the lateral walls of both fungiform and filiform papillae. "Collars" are raised structures associated with lateral walls of the papillae. These structures are prevalent on the tongues of the most sensitive human tasters, and on rabbits and monkeys. Diameters of papillae are associated with a higher prevalence of taste buds than are the heights of papillae. Individual subjects which have more fungiform papillae than their fellows also have more taste buds per papilla. Papillary shapes are more similar between humans and monkeys than between monkeys and rabbits. The number and location of taste pores on fungiform papillae of humans and rabbits change over time. Equivalent observations over time from the Cynomolgus monkeys are not currently available. These observations show that there are homologous attributes among fungiform papillae with taste buds on human, rabbit and monkey tongues.

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N.M. Targett. Allelochemical Cues in the Marine Environment: Understanding Interactions between Organisms and their Secondary Metabolites. University of Delaware, Graduate College of Marine Studies. Lewes, DE 19958.

Secondary metabolites have been implicated as putative cues in numerous interactions between and among organisms in the marine environment. Much of the work to date in marine systems identifies interactions mediated by secondary metabolites on the basis of the organism's behavioral response to the metabolite. There has been less focus on defining the physiological/ biochemical basis for the observed behavioral responses. In this presentation I focus on two examples from my own lab which seek to define the mechanisms by which animals respond to selected secondary metabolites. Specifically, I will address: 1. The response of herbivores to brown algal phlorotannins and 2. The response of predators to their terpene-rich gorgonian prey.

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"Marine Chemosensory Processes for Communication and Defense"

William Fenical, Scripps Institution of Oceanography , University of California-San Diego, La Jolla, CA 92093-0236

Chemosensory processes are pronounced activities which control prey selection, communication, and defense in competitive marine environments. While prey selection is normally conceived to arise from food components such as proteins and small peptides, communication and defense appear controlled by complex molecules produced by taxa-specific processes. An example in communication lies in the opisthobranch mollusk Navanax inermis, which produces alarm pheromones to signal danger. In defense, we now know that marine fishes use sensitive chemosensory processes to differentiate between palatable and unpalatable prey. In the Caribbean Sea, carnivorous wrasses avoid selected sea whips (soft-corals) due to their production of highly deterrent organic molecules of ornate structural forms. Examples of these adaptations and contrasts with analogous chemosensory processes in insects will be the basis of this presentation.

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TITLE: The Chemical Ecology of Plant-Insect Interactions
AUTHOR: Deane Bowers, Museum and E.P.O. Biology, University of Colorado

ABSTRACT

Plant secondary compounds (=natural products or allelochemicals) may be important in many aspects of the interactions of plants and their insect herbivores. Using my research on a particular group of these compounds, the iridoid glycosides, as an example, I will discuss some of the many roles that these compounds can play in plant-insect interactions. The underlying theme of this talk will be the importance of chemical variation for the plant and insect participants. I will focus on three different, but interrelated, questions: First, how does variation in iridoid glycosides determine hostplant suitability for insect herbivores? Second, how do insects respond to this variation? And third, what is the importance of this variation for the interactions of herbivorous insects and their predators?

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A Hyperpolarization-Activated Cation Conductance in Lobster Olfactory Receptor Neurons.
FRANK COROTTO AND WILLIAM MICHEL (Department of Physiology, University of Utah, Salt Lake City, UT).

The current underlying inward rectification in lobster olfactory receptor neurons was investigated using whole-cell patch clamp techniques. In current clamp, injection of negative current led to a hyperpolarization followed by a partial return (sag) towards the initial holding potential. In voltage clamp, hyperpolarizing steps elicited a slowly activating, non-inactivating inward current (I_h) that presumably underlies the sag observed in current clamp. Both the sag and I_h were blocked reversibly and specifically by extracellular application of 5 mM CsCl but were unaffected by 2 mM $BaCl_2$. I_h accounts for inward rectification in lobster olfactory receptor neurons; block of I_h by 5 mM CsCl caused the average input resistance to increase from 0.99 to 2.12 GΩ at hyperpolarizing holding potentials. The rate of I_h activation was voltage-dependent with time constants which ranged from 7.8 s at -69 mV to 248 ms at -114 mV. Analysis of tail currents revealed that the average reversal potential of I_h was 1.7 mV and was not affected significantly by a shift in E_{Cl}. The combined findings of Cs^+-sensitivity, Ba^{2+}-insensitivity, slow activation, and a reversal potential near 0 mV that is unaffected by E_{Cl} indicates that I_h is a hyperpolarization-activated cation current and not another type of inward rectifier. This current could play a number of roles in lobster olfactory receptor neurons including setting the resting membrane potential and input resistance, opposing hyperpolarizing inputs, and contributing to spike-frequency adaptation.

This work was supported by NIH DC0418.

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Specificity and Sensitivity of the Olfactory System of the Zebrafish, *Brachydanio rerio*. WILLIAM C. MICHEL, LARISSA M. LUBOMUDROV and AMY M. REED (University of Utah, Department of Physiology, Salt Lake City, Utah 84108).

In light of recent reports of zebrafish olfactory embryonic development and the similarity of zebrafish olfactory receptor genes with those of other vertebrates examined, it appears that this fish is emerging as an alternate model organism for olfactory investigations. To further our understanding of this olfactory system we have initiated an investigation of the olfactory specificity and sensitivity of adult zebrafish. Utilizing the electro-olfactogram recording technique we have established that the olfactory system is responsive to virtually all of the 24 amino acid and 12 bile acid stimulants tested. Typical of most teleost olfactory systems, the neutral and basic amino acids were more effective than the acidic amino acids. In general the long chain neutral amino acids were more effective than the short chain neutral amino acids. At both 10^{-4} M and 10^{-6} M the most effective bile acids (10^{-4} M taurocholic acid; 10^{-6} M glycocholic acid) were at least 50% more stimulatory than the most effective amino acids (10^{-4} M glutamine; 10^{-6} M leucine). The response thresholds for the more effective of the amino acid and bile acid stimulants were ca. 10^{-8} M and 10^{-10} M, respectively, and did not saturate at 10^{-4} M (the highest concentration tested). Comparison of the responses of female and male zebrafish to amino acid stimulants, suggest that females are more sensitive; however if the responses are standardized to 10^{-4} M cysteine, the relative effectiveness of the amino acid stimulants for both sexes are similar. Interactions between amino acid and bile acid stimulants were evident as an increase in the magnitude of the response to 10^{-4} M cysteine after exposure to a bile acid stimulant. The interaction is reciprocal; the magnitude of the response to bile acid stimulants was also increased after exposure to cysteine. Three steroids, β-estradiol, progesterone, and 4-pregnene-17α,20β-diol-3-one, were ineffective stimulants at both 10^{-4} M and 10^{-6} M.

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Electrophysiological Properties of Olfactory Receptor Neurons *In Vitro*. THOMAS C. BOZZA, DAVID P. WELLIS, DALE. D. HUNTER and JOHN S. KAUER (Neuroscience Program, Tufts University School of Medicine, Boston, MA 02111)

We are interested in studying the characteristics of olfactory receptor neurons (ORNs) as they differentiate from neuroblasts in the basal layer of the olfactory epithelium (OE). In order to perform electrophysiological investigations on individual cells, we have developed a short-term culture system (see also Pixley, 1992) in which neonatal rat olfactory epithelia are cultured with the C_6 rat glioma cell line. In dissociated cultures, ORNs ($NCAM^+$ process-bearing cells) survived for 7 days, frequently exhibited an asymmetric bipolar morphology, and extended long (>100μm), bifurcating processes. The death of these cells between 10 and 15 days *in vitro* is consistent with the behavior of axotomized ORNs. In order to ascertain whether ORNs expressed neuronal physiological characteristics, we made whole-cell patch clamp recordings from 32 ORNs cultured on C_6 glioma for 3 to 6 days *in vitro*. Typical resting potentials were near -40 mV and typical input resistances ranged from 2 to 4 GΩ. Small (10-30 pA) depolarizing pulses elicited single overshooting action potentials although multiple-spiking ORNs were also observed. Voltage clamp experiments revealed at least three distinct voltage-gated currents. These consisted of (1) a transient inward current (up to 2.5 nA), presumably Na^+ (2) a sustained outward current, presumably K^+ and (3) a transient outward current present in a majority of the ORNs studied. Spontaneous depolarizations resembling EPSPs were recorded in 4 cells raising the possibility that ORNs may form synapses with one another in these cultures. Combined with retroviral labeling *in situ*, this sytem might allow investigations into the electrophysiological characteristics of ORNs as they differentiate from basal cells.

Supported by a grant from the NIH.

Pixley, S.K. (1992) *Neuron* 8(6): 1191-1204.

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In Vivo Responses of Single Olfactory Receptor Neurons in the Channel catfish, *Ictalurus punctatus*. JIESHENG KANG and JOHN CAPRIO (Louisiana State University)

A large multigene family thought to encode odorant receptors was recently identified in the channel catfish (Ngai et al., *Cell* 72:657-666, 1993). In the previous report, *in situ* hybridization studies of the expression of receptor mRNAs in the olfactory epithelium suggested that a single olfactory receptor neuron (ORN) expressed only one or a few distinct odorant receptors. The present report is the first *in vivo* study of the responses of single olfactory receptor neurons (ORNs) in a teleost. The average (±s.d.) recording time of the 69 spontaneously active ORNs obtained from 22 preparations was 24±15 min. From a total of 303 stimulus presentations (excluding repeated trials), single ORNs responded to 38% of the tests with either a facilitation (13%; 39±18 a.p.s/5 sec.) or suppression (25%; 15±12 a.p.s/5 sec.) of activity; no significant change from spontaneous activity (23±12/5sec) occurred for 62% of the tests; however, individual ORNs responded to the different stimuli with various combinations of facilitory, suppressive and null responses. The percentage of facilitory, suppressive and null responses to the individual test stimuli across the ORNs tested were similar to that obtained from recordings of presumed mitral cells in the olfactory bulb of the same species (Kang and Caprio, in prep). All six of the stimuli [L-methionine, L-alanine, L-arginine, L-glutamate (each at 0.1mM), 1mM ATP or 0.3mM MBS (mixture of 3 bile salts)] were previously indicated to bind to independent receptor sites. For 37 ORNs that were each tested with the four amino acids (experiment A), 10 ORNs (27%) responded to one, 14 ORNs (38%) responded to two, 4 ORNs (11%) responded to three, and 1 ORN (3%) responded to four amino acids; 8 ORNs (22%) failed to respond. For 21 ORNs that were tested with the previous four amino acids and ATP and MBS (experiment B), 4 ORNs (19%) responded to only one, 6 ORNs (29%) responded to two, 4 ORNs (19%) responded to three, and single ORNs (5%) responded to 5 and 6 stimuli, respectively; 5 ORNs (24%) failed to respond. The responses were not correlated with stimulus quality. The results of both experiments (A and B) indicate that multiple stimulus receptor sites occur on individual ORNs.

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Sensory activation of the frog ciliated olfactory neuron Jean-François ROSIN, Tao JIANG and Didier TROTIER (EPHE, 1 av. des Olympiades, 91305 MASSY, France)

Using an *in vitro* electrophysiological approach, our aim was to characterize the reception and transduction steps successively involved in the elaboration of the olfactory sensory message.
Olfactory receptors molecules signal both the usual odorants and some agonists of neurotransmitter receptors with the same apparent low affinity. Apparent dissociation constants (K_d) for 1-butanol, carvone, IBMX, isoamyl-acetate and nicotine are higher than 10^{-6} M. Openings of ionic channels elicited by the intracellular injection of cyclic AMP ($K_d \approx 200$ μM) or cyclic GMP induced an inward current. The amplitude of the transducing current increased with the odorant stimulation intensity over a range of about one decade of concentration.
Beyond the amplification power of the metabolic coupling mechanisms, the exquisite sensitivity of the olfactory sensory neurons relies also on an efficient electrotonic conduction of the generator current between the apical end of the cell and the trigger zone at its basal pole. Estimated values of membrane resistance around the resting potential reached 20 to 30 GΩ. Small depolarizing currents not exceeding 1 pA, but lasting more than 100 ms, commonly triggered a single action potential. The resting potential is determined by a dynamical equilibrium established between a depolarizing conductance activated by the membrane hyperpolarization and an hyperpolarizing one activated by depolarization.
The basal rate of action potential emission by the recorded frog (*genus Rana*) neurons was less than 1 impulse.s^{-1}. The frequency of the action potentials was linearly related to the amplitude of a depolarizing transducing current. During current injection, the slope of this relation was close to 1 impulse.s^{-1}.pA^{-1}.

An Electrophysiological Survey of Frog Olfactory Cilia. STEVEN J. KLEENE, ROBERT C. GESTELAND, and SHIRLEY H. BRYANT (University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521).

Individual olfactory receptor neurons vary widely in their responses to odorants. Olfactory stimulus reception occurs in the cilia of the receptor neurons. Thus the variability among individual neurons could in part be due to differences among the olfactory cilia. We have quantitated the known conductance properties of each of 117 frog olfactory cilia. From a strictly qualitative viewpoint, the cilia were very homogeneous. All but a few of the cilia had a basal conductance in the absence of odorants and second messengers; conductances stimulated by cytoplasmic cAMP and by Ca^{2+}; and an adenylate cyclase that is stimulated by GTPγS. However, the magnitudes of the conductances varied widely among the cilia. Amplitudes of the cAMP- and Ca^{2+}-activated ciliary currents correlated strongly with one another across the 117 cilia and 24 frogs studied. This suggests that expression of the underlying channels may be co-regulated. None of the conductance properties correlated strongly with ciliary length, a marker of cell maturity. Given cytoplasmic MgATP as substrate, ciliary adenylate cyclase produced cAMP, which in turn gated membrane channels and increased the ciliary conductance. In some cilia, MgATP alone caused a very large increase in conductance. In others, there was little effect unless GTPγS, which increases cyclase activity, was also added. No effect of cytoplasmic IP_3 on ciliary conductance was detectable. Measured input conductances were corrected for the ciliary cable properties, assuming the cilium to be a cylinder of diameter 0.28 μm. In the absence of odorants and second messengers, the space constant λ for electrotonic conduction averaged 154 ± 15 μm (n = 100), which is several times longer than the average cilium. Addition of 300 μM cytoplasmic Ca^{2+} reduced λ to 35 ± 3 μm (n = 113). Cytoplasmic cAMP (100 μM) resulted in an average space constant of just 14 ± 2 μm (n = 117).

This work was supported by NIH grants PO1 DC00347, R55 DC00926, and R01 DC00926.

Odorants-induced hyperpolarizing K^+-currents in vertebrate olfactory neurons. MORALES, B., UGARTE, G., LABARCA, P., BACIGALUPO, J. (Dept. Biologia, Fac. Ciencias, Universidad de Chile & Centro de Estudios Cientificos de Santiago, Chile).

Olfactory transduction is thought to involve a cAMP cascade, which leads to a transient elevation of the action potential firing rate by activating a depolarizing inward current. However, a major fraction of odorants do not seem to activate the cAMP cascade. We compared the effect of a mixture of 3 of such odorants (mixture I: isovaleric acid, pyrazine and triethylamine) with that of a mixture of odorants that activate the olfactory adenylil cyclase on olfactory receptor neurons (mixture II: citralva, citronellal and geraniol). Olfactory receptor neurons were dissociated from the toads *C. caudiverbera* or *X. laevis*. The patch clamp technique was used either to monitor firing activity, or to record membrane potential or whole cell currents from these cells, while stimulating with controlled puffs of odorants directed to their ciliary regions. We found that stimulation with mixture I inhibited action potential firing in 10 of the 27 cells examined, having no effect on the rest of them. Five of the cells responsive to mixture I responded to mixture II with an increase in their firing rate. Mixture I triggered an outward current in 18 of 46 cells examined. This outward current was recorded from the same neurons that gave an inhibitory electrical response to mixture I. Within a wide voltage range (from -90 to +50 mV), the outward currents induced by mixture I did not reverse, whereas the reversal potential for mixture II-induced currents was found to be around 0 mV. The outward current induced by mixture I is highly selective for K^+. Furthermore, this current showed to be sensitive to TEA. We found that olfactory neurons in which mixture I triggered an outward current were hyperpolarized by the same stimulus under I-clamp. Our results show that certain odorants inhibit olfactory receptor neurons. In addition, they demonstrate directly that a same olfactory neuron has the ability to undergo excitatory and inhibitory responses, depending on the stimulus. Odorants that inhibit these receptor cells trigger an outward K^+ current, cause membrane hiperpolarization and inhibit action potential firing in olfactory neurons. Supported by Fondecyt 1930859 and 2930018.

Electrophysiological Responses to Carbon Dioxide from Olfactory Receptor Neurons in Mosquito Maxillary Palp Sensilla. ALAN J. GRANT, ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology) and BRUCE WIGTON (American Biophysics Corp.)

Each year, there are approximately 300 million clinical cases involving the five major diseases that can be transmitted by mosquitoes. Over 1.5 million fatalities can be attributed to these diseases, or their complications each year. For centuries, efforts have been made to better understand the mechanisms that underlie host attraction, feeding, and mating of hematophagous insects. A knowledge of the physiological capabilities of the sensory systems involved with the initiation and guidance of these behaviors is a prerequisite for the design of bio-rational methods of mosquito control. To date, little specific information is available which bears directly on this aspect of mosquito sensory physiology. We have recently completed an electrophysiological study of one of the three receptor neurons found in the basiconic sensilla on the maxillary palps of female mosquitos. This receptor neuron responds to very low concentrations (150-300 ppm) of carbon dioxide (CO_2), a stimulus known to be involved with host seeking behavior. We were particularly interested in evaluating the possibility that the detection of, or sensitivity to, step increases in CO_2 concentrations could be modulated by alterations in the background levels of CO_2 in a range which might be encountered during host-seeking behavior. We report here the concentration-response relationship for the CO_2 receptor neuron on the palps of female *Aedes aegypti* and the effects that different background CO_2 levels have on this relationship. In addition, several other species of mosquito including: *Anopheles stephensi*, *Culex quinquefasciatus*, *Culiseta melanura*, and *Aedes taeniorhynchus* were also tested to determine if the same physiological class of olfactory receptor neuron could be found in their basiconic sensilla. Preliminary studies were also conducted to determine the response specificity of the other two receptor neurons found in this class of sensilla to a range of specific compounds known to influence host-seeking behaviors.

Supported by NIDCD Grant DC00131

Dual IP3-gated Channels in Lobster Olfactory Receptor Neurons Have Different Ionic Selectivity and Display Kinetic Modes. FADOOL, D.A. and B.W. ACHE (Whitney Laboratory and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086).

Previously, we reported that inositol 1,4,5-trisphosphate ($InsP_3$) directly activates ion channels with two different conductances (30,74 pS) in the plasma membrane of cultured lobster olfactory receptor neurons. To further understand the role of one or both of these channels in excitatory transduction, we measured the ionic selectivity and gating properties of the channels. Two ion substitution paradigms were used to alter the reversal potential of 27 30 pS and 11 74 pS channels. With the calculated Nernst potential (E_x) for Cl^-, Na^+, or K^+ set away from 0 mV and that for Ca^{2+} set to 0 mV, 11 of 15 30 pS channels and 3 of 4 74 pS channels reversed near 0 mV, implying that the channels were either non-selective for cations or selective for Ca^{2+}. With E_{Ca} set away from 0 mV to discern between these alternatives, 12 of 12 30 pS channels continued to reverse at (n=7) or near (n=5) 0 mV, implying the smaller conductance channel is non-selective for cations. Under these conditions, six of seven 74 pS channels reversed at (n=5) or near (n=1) E_{Ca} (one continued to reverse at 0 mV), implying the larger conductance channel is more selective for Ca^{2+}. Both types of channels could be localized to the same cell. Both the 74 pS (n=8) and the 30 pS (n=11) channels displayed spontaneous changes in kinetics, entering two recognizable long open modes (Mode 1, $Pr_{open} \geq 0.91$; Mode 2, Pr_{open} = 1.0) and a closed mode that flickers to the open state (Mode 3, $Pr_{open} \leq 0.15$), in comparison to the most observed state (Pr_{open}= 0.27). Transitions from one mode to another were infrequent (highly diagonalized contingency tables, Chi-square, $p \leq 0.005$) and random (runs analysis), with sojourns in each mode ranging from 1-21 sec. In 16 trials, PKC and PKA significantly increased the Pr_{open} (n=7) and/or induced mode behavior (n=3), suggesting that phosphorylation state may favor mode transition. Both types of channels occur in the outer dendrite of the cells *in situ* (Ache et al., *AChemS* 1993) and presumably would be activated by odor-induced phosphoinositide hydrolysis. Our results suggest that $InsP_3$-induced Ca^{2+} entry and charge transfer for the odor-induced excitatory current may be mediated independently through separate effectors. Changes in the pattern of gating could influence the relative contributions of the channels to Ca^{2+} and cation entry. Analogous dual, plasma membrane effectors recently have been implicated in phototransduction in Drosophila (Hardie & Minke, *TINS* 16: 371, 1993).

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IP$_3$ and Cyclic Nucleotides Elicit Opposite Membrane Potential Changes in Squid Olfactory Receptor Neurons.

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Olfactory receptor neurons dissociated from the olfactory organ of squid, respond to the attractant, l-proline, with a depolarizing response, and to the aversive stimulus, dopamine, with a hyperpolarizing response. In an attempt to discover the transduction mechanisms underlying these changes in membrane potential, we included either 100 μM IP_3, 1mM cAMP or 1 mM cGMP in the patch pipette. We found that IP_3 activated a hyperpolarizing conductance that was not dependent on K^+ concentrations, but could be eliminated by shifting the Cl^- reversal potential from -90 mV to the cell's resting potential of approximately -50 mV. When cGMP was included in the patch pipette, 73% (8/11) of the cells depolarized by approximately 16 mV. cAMP also elicited a depolarization, however only 45% (5/11) of the cells responded to cAMP. Thus, these data reveal that at least two second messenger systems are present in squid olfactory receptor neurons, and suggest that the IP_3- and Cl^--dependent hyperpolarization underlies the response to dopamine, while the cyclic nucleotide-activated depolarization mediates the response to l-proline.

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Do olfactory neurons respond to odors with simultaneous activation of two second messenger pathways?

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Rapid kinetic experiments with isolated rat olfactory cilia have shown that odorants stimulate either cAMP production or an increase in IP_3 formation (Breer and Boekhoff, Chem. Senses. 16:19-29, 1991). However, experiments with embryonic rat olfactory neurons in culture show that some of the odorants used in the rapid kinetic experiments stimulate formation of both cAMP and IP_3 (Ronnett et al., J. Neurosci. 13:1751-1758, 1993). These results raise the question of whether intact single olfactory neurons respond to odorants through one or multiple second messenger pathways simultaneously. Olfactory neurons (ON) respond to odorants with an increase in intracellular calcium ($[Ca_i]$). Previous studies from our laboratory have shown that freshly isolated rat and human ON respond to odors that stimulate cAMP formation in rat olfactory cilia with an increase in Ca_i that is blocked by addition of the cAMP-gated channel antagonist l-*cis*-diltiazem (Restrepo et al., Biophys. J. 64:1961-1966, 1993, Restrepo et al., J. Gen. Physiol. 102:907-924, 1993). The present studies are designed to discriminate whether odorants stimulate one or both second messenger pathways in single isolated human olfactory neurons. We show that human ON respond to odorants that elicit IP_3 production in rat olfactory cilia, and that the increase in Ca_i produced by these odors is blocked by inhibiting IP_3 production with neomycin. In contrast, neomycin does not inhibit the response of ON to odorants that stimulate cAMP formation in isolated cilia.

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Localization of Inositol 1,4,5-trisphosphate Receptors in the Olfactory Neuroepithelium of the Rat and Channel Catfish.

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In earlier experiments we immunolocalized inositol 1,4,5-trisphosphate receptors (InsP3R's) to the olfactory epithelium of the rat using an antibody provided by Dr. T. Sudhof (antibody #T210; Mignery et al., Nature 342:192-195, 1989). Recently, we developed our own polyclonal antibody (Ab #2772) raised against a 19 amino acid peptide present at the C-terminus of the cerebellar type I $InsP_3R$. Western blotting using this Ab labeled a 120 kDa band in rat olfactory cilia, and a 107 kDa band in catfish olfactory cilia. Both bands had previously been identified as specific $InsP_3R$ binding sites by photoaffinity labeling with ASA $InsP_3$ (Kalinoski et al., Biochem. J. 281:449-456,1992 and Restrepo et al., Am. J. Physiol [Cell Physiol.] 263:C667-C673,1992). In the present study, we used immunohistochemical techniques to examine the distribution of $InsP_3Rs$ in the olfactory epithelium of the rat and channel catfish using AB 2772. In rat, immunoreactivity was specifically localized to the apical surface of the olfactory and respiratory epithelia, and labeling in the olfactory epithelium was greatly diminished in the ipsilateral side by unilateral bulbectomy. Using electron microscopy, Ab 2772 binding was localized to the cilia of olfactory neurons and microvilli of supporting cells although not all cilia present on the same olfactory neuron were labeled. In the catfish, immunoreactivity to Ab 2772 was found at the apical surface of subsets of neurons in the sensory epithelium by light microscopy. These results support previous evidence for a role for $InsP_3Rs$ in olfactory transduction.

This work was supported by NIH grant #DC-01228 and DC-00566.

Phospholipase C Gene Expression in Rat Olfactory Epithelium.

FE C. ABOGADIE, RICHARD C. BRUCH, ALBERT I. FARBMAN (Northwestern University) ROBERT WURZBURGER and FRANK L. MARGOLIS (Roche Institute of Molecular Biology).

The likely involvement of phosphoinositide-specific phospholipase C (PLC) in mediating olfactory responses to some stimuli has now been demonstrated in several species. However, no information is currently available regarding the identities and cellular localizations of PLC isotypes expressed in the olfactory epithelium. To investigate these questions, we used monoclonal antibodies to identify 3 PLC isotypes expressed in olfactory epithelium by Western blotting and to determine their cellular localizations by immunohistochemistry. PLCβ1 was found only in the glands below the epithelium in intact animals. However, following unilateral bulbectomy, PLCβ1 was expressed in receptor cells, but only in the endoturbinates on the contralateral side. PLCγ1 was found primarily in the apices of supporting cells, while PLCδ1 was found in the perinuclear region and dendrites of the receptor cells. PLC immunoreactivity was not detected in receptor cell cilia, suggesting that another PLC isotype is expressed in the cilia. To identify additional members of the PLC gene family expressed in the olfactory epithelium, degenerate primers were used in the polymerase chain reaction (PCR) following reverse transcription of total RNA. Several PCR products were obtained, ranging in size from about 150-500 bp, consistent with the conclusion that multiple PLC isotypes are expressed in olfactory epithelium. The PCR products were cloned into plasmid pCRII and are now being analyzed by automated DNA sequencing and Northern blots.

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Distribution and Phosphorylation properties of rat olfactory cyclic nucleotide-activated channel

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Odorant stimulation of olfactory sensory neurons results in membrane depolarization and firing of action potentials. This process is mediated by a G protein coupled cascade using cAMP as a second messenger. A cyclic nucleotide-activated channel thought to mediate olfactory signal transduction was cloned from rat cDNA library (Dhallan, et. al.) In order to characterize both the distribution of this channel in rat olfactory tissue and the signal regulation caused by channel phosphorylation, we developed an antibody against E.Coli expressed olfactory channel fusion protein.
Using immunohistochemistry, we could determine the distribution of the channel on rat olfactory tissue. In addition, western blotting could detect a 75kd band in rat olfactory tissue. By using primary cultures of rat olfactory receptor neurons, we could immunoprecipitate the 75kd protein as a phosphoprotein specifically in the presence of odorant. This data shows the possibility of regulation of olfactory signaling by channel protein phosphorylation.

Calcium Modulates the Rapid Kinetics of the Odorant-Induced Cyclic AMP Signal in Rat Olfactory Cilia

DONNA JAWORSKY (Johns Hopkins Medical Institution)
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Using a rapid quench/stop flow system, we have studied the effects of odorants on adenosine 3' ,5' -monophosphate (cAMP) production in rat olfactory cilia at millisecond timepoints, with longer times performed manually. The application of the odorants citralva, IVA, and IBMP elevates cAMP levels above basal. Cyclic AMP levels in cilia stimulated by these odorants peak at 100 ms and subsequently decline, indicating rapid activation and desensitization in the cilia. A more sustained signal is seen lasting 5-10 seconds after stimulation. Fold-stimulation over basal is dependent on free calcium concentration, showing an increase of basal and stimulated cAMP levels from 100 nM to 10 uM free calcium, and a decline in cAMP levels from 10 uM to 100 uM. All odorants tested were found to stimulate cAMP production, and the dose-response curves were multiphasic, with less stimulation seen at high concentrations. Complete dose-response curves for IVA were performed at varying free calcium concentrations, which show a change in magnitude of the curve peak and a rightward shift of the curve with increasing calcium concentration.

Follow-up Study: Efficacy of Group Therapy in the Treatment of Chemosensory Disorders.

ALAN R. HIRSCH (Smell & Taste Treatment and Research Foundation)
JONATHAN B. OSTER (University of Illinois Medical School)

We previously described group therapy in the treatment of chemosensory dysfunction (1). In that study improvement was seen in the Beck Depression Inventory, and analog scales of anxiety, anger, emotional acceptance and empathy. To assess the presence of persistent effects when treatment is terminated, all of those who underwent the above group therapy performed the Beck Depression Inventory and 25 questions about their feelings concerning their chemosensory dysfunction using a visual analog scale one month after termination of group therapy (2). Data was analyzed using the Wilcoxan non-parametric and Sign Rank Test. No statistical significance was found. During the post treatment month the following variables improved: analog scales of anxiety (4 of 7), anger (2 of 3), and life satisfaction (N=4). Variables that either remained the same or worsened included: Beck Depression Inventory (average = 3), analog scales of depression (2 of 4), acceptance (4 of 7), perceived empathy (3 of 4), emotional acceptance (N=3), mealtime problems (N=2), and sexual function (N=2). The above suggests that group therapy in treatment of chemosensory disorders should be considered as a method to evoke persistent benefits for psychological issues in those with chemosensory disorders.

1. Hirsch, A.R., Scott, J.M., Koch, S.H.: Efficacy of group therapy in the treatment approach to chemosensory disorders. In: Chemical Senses, 1992, 17:643.
2. Beck A.T., Ward, C.H., Mendelson, M., et al.: An inventory for measuring depression. Archives of General Psychiatry, 1961; 4:561-71.

Inhalation of Odorants for Weight Reduction.

ALAN R. HIRSCH (Smell & Taste Treatment and Research Foundation)
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Obesity is rampant in contemporary society. Surpisingly, despite the proliferation of methods for weight loss, no studies have been published which assesses the role of olfaction and weight loss. In order to do so, 3,193 volunteers at least 10 pounds overweight between the ages of 18 and 64, with no history of asthma, and not pregnant or breast feeding were recruited into this 6 month study. All subjects underwent olfactory testing with the Chicago Smell Test and the Thiophane Threshold Test of Amoore, rating of odor hendonics with visual analog scales, the Beck and Zung Depression Scales. The patient were weighed monthly and completed both Beck and Zung scales at the midway point and at completion of the study. The average age was 42.9 years with an average height of 64.7 inches and average initial weight was 216.9 lbs. The ideal weight based on BMI was 129 pounds. They were instructed to inhale odorants 3 times in each nostril whenever hungry. The use of inhalers varied from 18 to 285 sniffs per day. Average weight loss of 2.1% body weight per month (4.7 lbs.) was predicted by criteria including Amoore's Thiophane Threshold of 0 ($p<.05$), Chicago Smell Test identification of apple ($p<.05$), frequency of inhaler use ($p<.002$), medium or large body size ($p=.02$), not avoiding others ($p<.005$), not feeling badly about oneself ($p<.005$), positive hedonics for chocolate ($p<.008$), eating less chocolate bars ($p<.04$), eating more apples ($p<.06$), eating more mint candy ($p<.06$), eating an average of 2 to 4 meals per day, feeling bad about overeating, and snacking less than 5 times per day. This suggests the possibility that inhalation of odorants can induce sustained weight loss over a 6 month period.

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Diminished Taste Sensitivity in Patients Presenting to a Taste and Smell Clinic. B.J. COWART[1,2], I.M. YOUNG[2], R.S. FELDMAN[3] AND L.D. LOWRY[2] ([1]Monell Chemical Senses Center; [2]Jefferson Medical College; Veteran's Administration Medical Center, Philadelphia, PA)*

As others have also reported, at the Monell-Jefferson Chemosensory Clinical Center (MJC), we find only a small proportion of patients with chemosensory complaints to evidence measurable loss of sensitivity to tastes (49/581: 8.4%). As a result, there is still almost nothing known about this form of chemosensory dysfunction, and a preliminary description of patients with taste loss may be useful. Diagnosis of taste loss at MJC is based on measures of detection threshold for stimuli representing the four basic tastes. Of patients with threshold elevation(s), approximately half (24/49) evidenced loss in sensitivity to a single tastant, and only 4 evidenced diminished sensitivity to all basic tastes. Taste loss was rarely observed in the absence of phantogeusia and/or smell dysfunction (7/49); in fact, over ⅓ of our patients with taste loss reported a phantom taste (17/49). Among patients with a quality-specific loss, salt sensitivity was most frequently affected (50%), followed by sour (25%), bitter (16.7%) and sweet (8.4%). Moreover, a loss in sensitivity to salt, singly and in combination with other tastes, was more likely to be associated with phantogeusia than other kinds of loss ($p<0.03$). Taste loss patients tended to be older than patients with smell dysfunction alone ($p<0.02$); they were also more likely to report weight loss as a result of their chemosensory disorder ($p<0.001$). Finally, the etiologies of taste losses are more often obscure than is the case with smell losses, although more than half of the cases involving loss in sensitivity to multiple tastes seemed to be associated with either prior upper respiratory infection or head trauma.

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What do the community-dwelling elderly know about chemosensory loss and how do losses affect their behavior?
MARCIA LEVIN PELCHAT, MONICA FIRELY, ROLAND SOTELLO & ALVIN OUTLAW (Monell Chemical Senses Center).

The aims of this study were 1) to assess the effects of age-related olfactory loss on lifestyle and quality of life and 2) to identify needs for educational intervention. Eighty young (18-35 y.o.) and elderly (65-87 y.o.) adults underwent olfactory threshold and odor identification testing and filled out a questionnaire. Based on age and olfactory function, they were divided into three groups: young (Y), old with good olfaction (OG), and old with poor olfaction (OP). The Y and the OG groups were matched on olfactory function scores. As reported in previous studies, there were no age or olfaction-related differences in self-reports of eating enjoyment. Elderly subjects with poor olfaction reported greater enjoyment of dried fruit and of grapefruit than did members of the other groups. However, this difference did not extend to all fruits. There were no other trends for differences in preferences for general categories of food (e.g. spicy, crunchy, salty) or for specific foods as a function of olfaction. There were, however, several age cohort distinctions: Young subjects reported greater preferences for spicy and ethnic foods as well as for "junk foods" such as gum or french fries. There was little concern by subjects in any group about ability to detect household dangers such as smoke or gas leaks. There is clearly a need for education on this topic. Subjects in the OP group were significantly more concerned about the possibility of wearing too much fragrance and tended to use fragrance less frequently than others. There were no olfaction-related differences in preferences for scented vs. unscented products such as detergent or deodorant; however, young subjects tended to prefer scented products more than elderly subjects did. Most subjects, particularly the elderly, seemed to be unaware that olfaction plays a role in the discrimination of flavor. This is another area for educational intervention.

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Effects of Traumatic Brain Injury on Olfactory Function in Children.
RANI NIJJAR (San Diego State University), JUDITH ANDERSON (UCSD Medical Center), KRISTEN KONAR (San Diego State University) and CLAIRE MURPHY (UCSD Medical Center and San Diego State University)*

Traumatic brain injury (TBI) is the predominant childhood accident and the leading cause of childhood death and disability, with 200 of every 100,000 children in the United States sustaining TBI each year. Smell loss is frequently overlooked during clinical evaluation following head trauma, although, loss of olfactory function is estimated to occur in 5% to 30% of all patients with head trauma, with incidence of anosmia increasing with the severity and locus of injury. These deficits have not been well characterized to date in children. The present study looked at both olfactory thresholds and odor identification in children with mild to severe TBI within two weeks to one month post injury. A total of 49 subjects participated: 24 head-injured patients (Mean age = 10.2), and 25 controls (orthopedic and non-injured subjects) (Mean age = 9.7). Glascow Coma (GC) scores were obtained in order to rate the severity of the head trauma. GC scores range from 1 (severe trauma) to 15. Subjects were divided into two groups based on scores on the GC. Subjects with scores of 11 and above were considered to have mild to no trauma and subjects with scores of 10 and below were considered to have moderate to severe trauma. Patients were excluded from the study if they had history of other known neurologic disease (e.g., chronic medical illness, mental retardation) prior to head injury. The results indicated that children with head trauma had significantly poorer odor threshold sensitivity and odor identification ability than normal controls, $p < .05$. In addition, patients with moderate to severe head trauma had significantly poorer odor threshold sensitivity than those with mild to no head injury, $p < .05$. The findings of this study suggest that olfactory function is an important factor that should be included in the clinical evaluation of childhood head trauma.

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Reliability of the Odorant Confusion Matrix: Test-Retest
DANIEL B. KURTZ, PAUL R. SHEEHE, PAUL F. KENT, THERESA L. WHITE, DAVID E. HORNUNG, HERBERT N. WRIGHT* (Smell and Taste Disorders Clinic, SUNY Health Science Center, Syracuse 13210)

This study evaluated the test-retest reliability of the Odorant Confusion Matrix (OCM) (Wright, 1987) in a clinical population. In the OCM, patients identify, from a list of 10 odorant names, each of 10 odorant stimuli presented in 10 randomized blocks. A simple index of olfactory performance is given by the overall percent correct. To evaluate the reliability of the OCM, thirteen hyposmic patients seen at the Smell and Taste Disorders Clinic agreed to be retested within 2 hours of their original OCM. Average percent correct on the retest was not significantly different than the original test (test=42.8%, retest=44.2%, paired t-test, p=n.s.) and the test/retest scores were highly correlated (r^2=.832). Since each odorant is presented 10 times, the OCM results in a pattern of odorant misidentification. To explore the test-retest reliability of the patterns of odor misidentification, the dissimilarity between the test and retest OCMs produced by each patient was determined. The dissimilarity between each patient's OCM and the test and retest OCM of every other patient produced a 26x26 dissimilarity matrix. The dimensionality of these dissimilarities was reduced and displayed by multidimensional scaling (MDS). This technique arranged the patients' test and retest in 5-dimensional space such that those OCMs which were less dissimilar were closer together and those which were more dissimilar were further apart. Dimension 1 was highly correlated to overall performance on the OCM (percent correct). Across all dimensions, the retest was generally closer to the test of the same patient than to either the test or retest of other patients, and movement in Dimension 1 (variation in percent correct) was similar to the movement in Dimensions 2 - 5. That is, the consistency of the pattern of responses appears to be similar to the consistency of overall percent correct. The stability of these patterns is crucial for the OCM to be useful in identifying particular etiologies of olfactory dysfunction.

*Deceased

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Odor Memory and Learning in Healthy Elderly vs. Young Adults: Recall, Recognition Memory, and Identification. LETICIA ACOSTA, CHARLIE D. MORGAN (San Diego State University), STEVEN NORDIN and CLAIRE MURPHY (UCSD Medical Center and San Diego State University)

Short- and long-delay recall for odors are highly demanding cognitive tasks since they, besides encoding, storage, and retrieval, also require correct identification. It can therefore be hypothesized that normal aging and, even more so, dementia would have adverse effects on odor-recall performance. Since, to our knowledge, odor recall has not been characterized in the elderly, our first step was to study aging effects on short- and long-delay recall and recognition memory for odors and verbally presented words, the latter modality for comparison. This was accomplished by giving young adults and healthy elderly adults (screened for dementia) the California Verbal Learning Test (CVLT; Delis et al., The Psychological Corp., 1983) as well as an analogous test that applied odors. More specifically, the CVLT provides information on functions such as short- and long-delay recall, serial recall strategies, position recall strategies (primacy and recency effects), learning effects over trials, and recognition and response bias. Moreover, for the odor test, free identification was finally assessed to determine what role identification vs. memory played on recall performance. The odorants used were among the most identifiable (from a battery of 80 odors) for healthy elderly subjects and matched in familiarity with the verbal words used in the CVLT, enabling a direct comparison between tests. The results demonstrated significantly poorer short- and long-delay recall ability, learning over trials, and long-delay recognition memory in elderly than in young for both odors and verbally presented words. Interestingly, the difference in short-delay recall ability, learning over trials, and recognition memory between groups was larger for odors than for verbally presented words. A poor identification ability was found in the elderly that accounted for a large proportion of the poor recall performance. The present findings may prove to be valuable for the development of a standardized odor-recall test, which may be a useful tool for testing olfaction in demented populations.

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Odor Detection Threshold is Impaired in Patients at Risk For Dementia. L. JILL RAZANI (SDSU-UCSD Joint Doctoral Program), STEVEN NORDIN, and CLAIRE MURPHY (UCSD Medical Center and San Diego State University)

Alzheimer's Disease is a debilitating progressive brain disorder that is only definitively diagnosed on autopsy or biopsy. Tremendous effort has been made in recent years towards the development of treatment strategies for dementing illnesses. Procedures for definitive differential diagnoses are desperately needed so that patients can receive appropriate medical care once treatment is available. Previous research has clearly demonstrated impairment in odor identification ability and odor detection sensitivity in patients with moderate to severe Alzheimer's dementia. These studies suggest that olfactory sensory tests may aid in the diagnosis of Alzheimer's Disease. However, it is unclear whether the sense of smell is impaired in patients in the very early stages of dementia. In the present study, odor detection thresholds of patients at risk for dementia and normal age and sex matched controls were examined. Patients' cognitive functioning was assesed using the Fuld (1978) adaptation of Information-Memory-Concentration test of Blessed, Tomlinson, and Roth (1968). Subjects with scores of 3 and above on this exam were placed in the at risk for dementia group. A two-alternative, forced-choice, ascending method was used for assessing odor detection threshold for the odorant butanol. Incorrect choices led to stronger concentrations and correct choices led to presentation of the same concentration, to a criterion of 5 successive correct responses. Each nostril was tested separately and the average of the two nostrils was used for analysis. The results of this study showed that odor detection sensitivity was significantly poorer for the at risk for dementia patients than the normal controls. These findings suggest that perhaps the CNS regions involved with olfactory-mediated tasks are affected from the very early stages of dementia and that tests that measure odor sensitivity may be very useful for assisting in the diagnosis of dementia.

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Recognition Memory for Odorants and Visual Stimuli in Elderly at Risk for Alzheimer's Disease: A Comparison with Patients with Probable Alzheimer's Disease and Normal Elderly. JODI HARVEY (San Diego State University), STEVEN NORDIN and CLAIRE MURPHY (UCSD Medical Center and San Diego State University)

Elderly at risk for Alzheimer's disease (AD) is a desirable population to study because of the information of early signs of the disease they may provide. A large number of studies suggest marked changes in olfactory functions in probable AD, and the question of whether olfactory tests can be used for obtaining early markers of the disease has been raised by several researchers. The present study compared eight elderly at risk for AD with 71 probable AD patients and 82 healthy elderly controls with respect to short-term recognition memory for odorants and visual stimuli. All subjects were diagnosed by two independent neurologists at the UCSD Alzheimer's Disease Research Center. All odorants used were common household products (e.g., baby powder, cloves, honey). The visual stimuli (faces of presidents and vice presidents, engineering symbols, and colors) were used as comparison modalities to determine whether a potential decline in memory for odorants was specific to olfaction per se, or whether it was more global in origin. Ten stimuli of each modality were initially inspected by the subject, followed by five old and five new stimuli to be evaluated as either old or new. The results showed that the elderly at risk for AD performed more poorly than the controls, but better than the probable AD patients for all stimulus modalities. No significant interaction between group and stimulus modality was found, suggesting that recognition memory for odorants and visual stimuli does not differ in disparity between groups. However, the memory performance for odorants, but not for visual stimuli, in the probable AD patients was found to correlate weakly but significantly with degree of dementia (Dementia Rating Scale; $r = 0.28$, $p < 0.05$). In conclusion, the poor odor-recognition memory in the elderly at risk for AD in the present study is consonant with findings that short-term recognition memory is affected in AD at an early stage, and that performance on recognition memory for odorants in specific is related to the progression of the disease.

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Odor Pleasantness Ratings Predict Food Preference Patterns. BRYAN RAUDENBUSH, PAUL FLASPOHLER & ROBERT A. FRANK (University of Cincinnati, Cincinnati, OH).

The relationship of odor pleasantness ratings and food preference patterns was assessed. Subjects (N=101) were asked to make pleasantness ratings of 10 food odors (pineapple, coconut, coffee, lemon, bouillon, butterscotch, blueberry, onion, asparagus, clam chowder) on an 11 point category scale from not pleasant (0) to very pleasant (10). They also completed the Food Attitudes Survey (FAS) which measures food likes, dislikes and willingness to try 217 common, unusual and fictitious foods, beverages and condiments. In addition, subjects completed the Activity Attitudes Survey (ACT), Sensation Seeking Inventory, and Optimism/ Pessimism Scale. The average pleasantness ratings across odors were significantly correlated with food likes (r=.37), dislikes (r=-.28) and the number of foods subjects were unwilling to try (r=-.33). The average pleasantness ratings were not found to correlate significantly with the other personality measures, with the exception of number of dislike activity responses (r=-.29). The same correlational patterns emerged when partial correlations were computed between average pleasantness ratings and likes, dislikes and won't trys controlling for gender, age, and ACT, Sensation Seeking and Optimism/Pessimism scores. Thus, odor pleasantness ratings were specifically predictive of food preference patterns, and were not predictive of activity preference patterns, sensation seeking or optimistic/pessimistic personality.

A Smell Test Based on Odor Cognition of Japanese People

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SAHO AYABE (Inst. of Psychology, Univ. of Tsukuba)
SHINJI SATOH (Dept. of Medicine, Univ. of Tsukuba)

Odor cognition might be formed by perceptions of one's personal experience from the environment. We have done many experiments about odor detection, identification, classification and memory. Based on these data, about 20 odors have been selected as a representative of typical odors for Japanese people. This contains special odors for the Japanese like to *miso-soy, laver(marine plants), Japanese orange, and India ink.* These odors are micro encapsulated and printed on a paper card as a smell test (STAUTT; Smell Test of AIST, University of Tsukuba, and Takasago Int. Corp.) for Japanese people. The stability of this test was examined and several experiments were tried for aged persons.

Odor substances were offered by Takasago Int. Corp..

Relative Independence of Odor Quality Discrimination and Odor Detection in Aging. RENE A. DE WIJK (J.B. Pierce Laboratory & Yale Univ.), MARIA NORDIN (San Diego State Univ.), WILLIAM S. CAIN (J.B. Pierce Laboratory & Yale Univ.), STEVEN NORDIN & CLAIRE MURPHY (UCSD Medical Center & San Diego State Univ.)

Our previous research with temporal-lobe epilepsy patients indicated that central olfactory impairment may reveal itself in losses in quality discrimination quite apart from changes in absolute sensitivity. We have explored this topic further in a study of normal aging that included 16 young adults, 16 middle aged adults, and 16 elderly adults. The question was whether aging might also lead to alterations of quality discrimination over and above any threshold changes. Subjects were tested for 1) threshold sensitivity (4 tests for high reliability), 2) odor quality discrimination (ABX design with 2 series of odors) and 3) visual pattern discrimination. We anticipated that both threshold and odor discrimination would show an age-associated decline, as indeed they did. So too did the visual discrimination. For odor discrimination, we tested subjects with both a fixed level of intensity and a level chosen to compensate for differences in threshold. Age-related differences in odor discrimination persisted despite attempts to normalize intensity by compensating for sensitivity. Furthermore, analysis of covariance indicated that neither threshold nor performance at visual discrimination, both of which correlated with odor discrimination, could account entirely for the age-associated differences in discrimination. We conclude that in aging, as in some pathological conditions, quality discrimination can suffer losses independent of other aspects of performance.

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The Conditioning of Anxiety to an Odor: A Possible Mechanism of Symptom Maintenance in "Sick Building Syndrome." **PAUL D. NEWMAN, LLOYD HASTINGS & ROBERT A. FRANK (University of Cincinnati).**

Investigations of "sick building syndrome" (SBS) often fail to identify any causative agent. However, the occurrence of a non-toxic novel or unique odor is often found to be associated with such incidences, raising the possibility that odor conditioning plays a role in SBS symptoms. We set out to determine if anxiety, produced by a difficult cognitive task, could be conditioned to an odor, such that the odor alone becomes sufficient to produce an increase in anxiety. The Stroop Color-Word task was used to create an anxious state in 42 undergraduate psychology students. Twenty-one of the subjects were exposed to the odor 1-butanol at a concentration of 150 ppm during the task, while the other subjects received just air. After a rest period, the subjects' state-anxiety levels were measured using the State-Trait Anxiety Inventory (STAI). All subjects were then exposed to the 1-butanol for 5 minutes after which their state anxiety levels were again measured. When subjects with a compromised sense of smell (reports of nasal allergies, being "stuffed up," etc.) were eliminated, the conditioned group was found to have a significant increase in anxiety upon the second administration of the odor, while subjects not previously exposed to the odor did not show an increase in anxiety. In addition, the final level of anxiety was significantly higher in the conditioned group than in the non-conditioned group. These results support the possibility that an odor associated with a stressful situation can induce anxiety when presented alone at a later time. Likewise, such findings are consistent with the idea that SBS symptoms may be maintained by the existence of an odor, previously associated with a stressful environment.

Effects of Labeling on the Perception of Fat in Foods and Its Relationship to Preference. BEVERLY J. TEPPER and SUSAN E. SHAFFER (Rutgers University).

In most psychophysical studies, samples are evaluated blind whereas, in the real world, foods are labeled with calorie and nutrient content. This study explored the extent to which labeling influenced attribute ratings of two foods, and whether preference for fat content is related to perception of these attributes. Our previous work (unpublished) suggested that individuals who preferred a low-fat (LF) version of pudding, were unable to distinguish a high-fat (HF) versus a LF version in a blind, paired comparison test. Here, 230 young adults tasted two samples of vanilla pudding (2.5% or 10% fat) and chex mix snack (~20% and 45% fat). Samples were prepared in the laboratory to be visually similar. Half of the subjects received samples labeled as LF or regular version and half received coded samples. Subjects rated perceived creaminess (pudding), crispness (snack) and fat content (both foods) using 15 cm line scales. Subjects also chose the sample they preferred. Subjects did not perceive differences in crispness in the snack although they did make these distinctions in creaminess in the pudding ($p \leq 0.001$). The presence of the labels did not change these perceptions. Subjects perceived the LF versions of the foods to be lower in fat content ($p \leq 0.001$). As expected, the labels magnified these differences. Individuals who preferred the LF pudding were unable to differentiate creaminess of the samples if they were unlabeled. This suggests that sensory judgements (creaminess and crispness) were resistent to the presence of labels, but ratings of fat content, a more cognitively based decision were more sensitive to labeling. Those who preferred the LF version (fat-dislikers), apparently could not perceive differences in the creaminess of the samples. Whether the relationship between perception and preference suggests real individual differences in the sensory perception of foods or whether fat dislikers are merely less attentive to perceptual differences between foods requires further study.

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Odor Memory Shows Directed Forgetting Effects

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The authors gratefully acknowledge the support of the William H. Wheeler Center for Odor Research for the funding of this study.

Odors seem to be treated differently than other stimuli by the memory system. Odor memory has been shown to be less effective than verbal or visual memory (Engen, Kuisma, & Eimas, 1973), yet the recognition accuracy for odors shows little sign of decay over time (Engen & Ross, 1973; Lawless & Cain, 1975). The study presented here extends our consideration of the nature of forgetting in odor memory. Odors seem to have a unique forgetting function, low initial performance with less forgetting over time. We were interested in determining if this meant that tasks shown to enhance forgetting of verbal material would have no effect on odors. The directed forgetting procedure reliably produces forgetting of verbal stimuli (MacLeod, 1989). Directed forgetting occurs when subjects are presented with a stimulus, then instructed to forget that item or to remember the item for a subsequent memory task. Forget items are recognized less than remember items. We designed the study reported here to examine the existence of directed forgetting in olfactory memory. A pilot study had indicated that we would observe directed forgetting but a central concern was that subjects might be forgetting the names of the odors rather than the odors themselves. To allow a consideration of this issue, we executed a mixed factorial design which crossed Encoding (3) X Testing (2) X Instruction (2). Subjects were presented at Encoding with either a set of 60 odorants, the labels for the odorants, or both the odorants and the labels. At the time of Testing, subjects were tested for the old-new recognition of either the odorants or the labels. Nested within the encoding variable was the instruction for directed forgetting, half of the items were indicated as forget items and half were designated remember items. Directed forgetting was observed in every condition of the study, but did not interact with the Encoding or Testing variables. A 3X2X2 repeated measures analysis of variance demonstrated this effect. There was a significant main effect of instruction, $F(1,54) = 12.42$, $p < .001$, but no interaction with the remaining variables. This study demonstrates that the recognition of odors can be inhibited. Further, we have shown that the effect cannot be accounted for solely by the modification of label memory.

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Novelty and Context Determine Odor Retrieval Cue Effectiveness.

RACHEL S. HERZ (University of British Columbia).

In both humans and animals it has been shown that when an odor is novel the circumstances and context surrounding its first experience will determine the responses and associations that are later made to it. Thusly, it was proposed that in order for an odor to be an effective memory cue it should be novel at the time it was first experienced in conjunction with a learning event. Additionally, the distinctiveness of the odor should also determine its later efficacy as a retrieval cue. To test this proposition an experiment with human subjects was conducted in which three different ambient odor conditions which varied in familiarity and contextual relevance and a no-odor control condition were compared (N= 16 in each condition). An average level of odor intensity was used, with specific odor concentrations varying depending on the odorant tested. Ambient room scent was achieved by passive diffusion of the odorant from several fixed sources in the room. The results revealed that when subjects incidentally learned a list of words in the presence of an unfamiliar ambient odor (osmanthus) and were tested 2 days later for their memory of those words with the same odor present, they recalled more words than subjects in any of the other three conditions. Furthermore, subjects recalled more words in the presence of a familiar odor that was not contextually relevant to the laboratory (peppermint) than a familiar odor that was contextually relevant to the laboratory (pine cleaner). Indeed, recall in the presence of the contextually relevant odor was no better than with no odor present. It has previously been shown that an ambient odor can serve as an effective retrieval cue (e.g., Schab, 1990). The present results now demonstrate that the familiarity and the contextual relevance of an ambient odor are important determinants for cue effectiveness. This finding supports the theory that odor novelty and distinctiveness are important for forming olfactory associations in memory.

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Sensitivity to Warning Agents for Natural Gas. WILLIAM S. CAIN, J. ENRIQUE COMETTO-MUNIZ, ROBIN R. BABBITT, & JANNEANE F. GENT (John B. Pierce Laboratory and Yale University).

Manufacturers of warning odorants offer more than a score of products considered suitable for perception of natural gas leaks. Most products are blends that contain some fraction of the powerful odorant t-butyl mercaptan (TBM) and a second component such as dimethyl sulfide (DMS), methyl ethyl sulfide (MES), or tetrahydrothiophene (THT). The choice of a particular blend depends in part on the climate in the region served by a gas company, though the choice is never completely determined. The choice of level of odorant to add also depends in part on climate, but is modulated by experience with complaints about leaks in the field and is partly a matter of traditional practice. Most gas companies can anticipate that the amount of odorant they add will meet the requirement of making the gas detectable by distinct odor at a level of at least one-fifth the lower limit of flammability. They may not know how many people with weakened olfaction their choice of agent and level leaves unprotected. In terms of number of people affected, the elderly comprise the principal group at risk for weakened olfaction. We studied the relative abilities of groups of approximately 90 young and elderly subjects to detect (by a 90% criterion) various blends and their ingredients. Within both young and elderly groups sensitivity varied more than a hundredfold, irrespective of agent. Sensitivity of the median elderly person, however, usually fell within a half log unit of that of the median young person. The outcome implied little variation in the size of the gap from agent to agent. Nevertheless, a small subset of the elderly group proved surprisingly refractory, an outcome that highlights the need for clearer delineation of risk factors for olfactory loss among the elderly. With respect to the understanding olfaction per se, a blend of TBM and DMS proved intriguing. It showed considerably more potency vís a vís its ingredients than any theories or models of how humans detect mixtures would predict.

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Masking Is a Matter of Perceived Intensity of Masker. EDWARD MONAHAN, WILLIAM S. CAIN, & MATS J. OLSSON (John B. Pierce Laboratory and Yale University).

The control of malodors via introduction of other more acceptable odors takes advantage of two basic olfactory phenomena, hypoadditivity in mixtures and masking or alterations of one quality by another. The rules of hypoadditivity seem relatively similar from one pair of odorants to another, e.g., a mixture of two equally intense stimuli will typically smell only about 20 to 30% stronger than either alone. The rules of masking are less clear, though in laboratory situations where subjects have been given some practice with estimating the intensity of the perceptual "components" of mixtures, one pair of compounds behaves much like another. In particular, masking seems symmetrical, e.g., for comparable perceived intensities A masks B to about the same degree that B masks A. Field experience would suggest, however, that some substances have special properties as maskers or as difficult-to-mask compounds. One possible reason that laboratory data have not uncovered such differences is that investigators have not studied some of the more interesting compounds. In the present study, we chose a substance notoriously difficult to mask [thioglycolic acid (TGA)] and paired it with various possible maskers (cinnamon oil, ambrettolide, trimofix, peppermint oil, and citronalva) in a relatively typical study of odor mixtures and masking. The results showed that different maskers had approximately the same masking effect on TGA. Moreover, the perceived proportion of TGA in a mixture of equally strong components was typically close to 0.5. According to recent research, this is commonly the result when combining any two odors. These results suggest that TGA is not harder to mask per se, but that its residual quality is simply poorly accepted. The results add to an emerging picture that the masking ability of an odor lies primarily in its perceived intensity rather than in its quality.

Effect of Attention on the Chemosensory (Olfactory) Event-related Potential in Humans.

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Event-related potentials (ERP's) were recorded from two groups of normosmic, young human subjects (age 20-35 years). In Group I (n=4), odorant stimuli were delivered at a constant interstimulus interval of 18 s, and the subjects were required to indicate detection of the stimulus by pressing a button. In Group II (n=5), odorant stimuli were delivered at variable interstimulus intervals ranging from 6-30 s, and no behavioral response was required. Otherwise, the same stimulus was used for both groups consisting of 50% amyl acetate, 40 ms duration, at a flow rate of 5 L/min presented monorhinally through a nasal cannula.

Evoked potentials from the two groups of subjects showed similar morphology. The waveform consisted primarily of a negative component (N1) and a positive component (P2) at all electrode sites. Peak latency and amplitude measures from the subjects in each group were subjected to analysis of variance. Significant differences between the groups were seen only at the Fz electrode for N1 latency (p=0.03) and N1/P2 peak-to-peak amplitude (p=0.05). This finding suggests that attention to the odorant stimulus results in a change in the topography of the chemosensory ERP, with a relative increase frontally in the amplitude of the primary components associated with an earlier onset of the N1 component in this region.

Initiation and Termination of Second Messenger Signaling in Olfactory Neurons. BREER, H., BOEKHOFF, I., SCHLEICHER, S. and E. TAREILUS (Institute of Zoophysiology, University Stuttgart-Hohenheim), 70599 Stuttgart, FRG

Odorants are detected by chemosensory neurons which encode the strength, duration and quality of odorant stimuli into afferent neuronal signals. A large variety of receptor-subtypes is supposed to recognize and discriminate odorous molecules; heterologous expression experiments have indicated that distinct receptor types may be tuned to interact with a rather broad spectrum of odorants. Upon interaction of odorous molecules with specific receptors, second messenger cascades are activated in a G-protein dependent manner leading to a rapid and transient signal of cAMP or IP_3. These second messenger 'pulses' are supposed to elicit the generator current via direct gating of cation channels. The characteristic phasic response of olfactory neurons is due to a rapid termination of the odor-induced primary reaction; response termination is important for resetting the cell so that it can respond to repetitive stimuli. Rapidly 'turning off' the odor-induced second messenger signalling cascade is accomplished by uncoupling the reaction cascades via a negative feedback reaction. This 'turn off' reaction is mediated by a sequential interplay of two types of kinases: a second messenger activated kinase and a ß-adrenergic receptor kinase-like enzyme. These kinases lead to a stimulus-dependent phosphorylation of olfactory receptor proteins.

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Does Nitric Oxide Participate in Olfactory Transduction of L-arginine in Sea Lamprey Larvae? B.S. ZIELINSKI (Univ. of Windsor, Canada), T.J. HARA (Freshwater Institute, Dept. of Fisheries and Oceans, Winnipeg Canada), J.K. OSAHAN, E. WONG and M. HOSSEINI (Univ. of Windsor, Canada).

Sea lampreys represent an ancient evolutionary line of vertebrates, and possess a well-developed monorhinal olfactory system. In larval sea lampreys, L-arginine was a potent stimulant when EOG responses were recorded from the posterior surface of the olfactory organ where ciliated olfactory receptor cells are located. We have investigated the possibility that the L-arginine response is mediated by nitric oxide (NO), a signaling molecule produced from L-arginine by the calmodulin dependent enzyme, NO synthase. N-monomethyl-L-arginine, a blocker of NO synthase competitively inhibited the EOG response. We localized NO synthase in the dendrites and olfactory knobs of olfactory receptor cells by light and electron microscopic examination of tissue reacted by the NADPH diaphorase technique. Histochemical specificity for NO synthase was demonstrated by enhancing the labeling with L-arginine, calcium and calmodulin, and by diminishing the staining intensity following inhibition of NO synthase with N^G-nitro-L-arginine. We conclude that in sea lamprey larvae, NO mediates olfactory receptor cell responses to L-arginine.

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Nitric Oxide Expression During Olfactory Neuron Development And Regeneration

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Nitric Oxide (NO), which is produced by the enzyme Nitric Oxide Synthase (NOS), has been implicated as a neuronal intracellular messenger in the mature central and peripheral nervous systems. NO is capable of activating guanyl cyclase to induce cyclic GMP (cGMP) production. In the mature olfactory receptor neuron, the cyclic nucleotide gated channel appears to be more sensitive to cGMP than cAMP. Using an antibody generated against a fusion protein of neuronal NOS, we have examined the expression of NOS in the rat olfactory system during development and also following bilateral and unilateral bulbectomy. Although NOS is not present in the mature olfactory neuron, it is highly expressed in the nervous layer of the olfactory placode as early as E11-12. Embryonic olfactory neurons are highly immunoreactive for NOS in cell bodies, developing sensory cilia and within afferent projections to the developing olfactory bulb. By E19, immunoreactivity decreases and is confined to olfactory neuron axonal projections. Olfactory neuron NOS expression declines rapidly after birth and is undetectable by P7. Following bulbectomy, NOS expression is rapidly induced in the axonal projections of regenerating olfactory neurons. Western and Northern blot analysis support immunohistochemical observations, demonstrating an increase in NOS protein and mRNA by 3 days and 1 week post-bulbectomy, a transient decrease at 2 weeks post-bulbectomy and a subsequent increase at 3 weeks post-bulbectomy. These data correlate with established "waves" of regeneration within the target-deprived olfactory epithelium and support a role for NO in activity-dependent control of olfactory neuron development.

Metabolic Activation Of A Potent Olfactory-Specific Toxicant, 2,6-Dichlorobenzonitrile (DCBN), By P450 2As.

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DCBN is a widely used herbicide that has been shown to cause tissue-specific toxicity in the olfactory mucosa of rodents at very low doses. The toxicity of DCBN is reportedly P450-dependent, although the isozymes involved have not been identified and the effects of DCBN on humans are not known. In the present study, DCBN metabolism was examined with microsomes or with purified P450 in a reconstituted system. Rat and rabbit olfactory microsomes metabolized DCBN to an epoxide intermediate that forms covalent adducts with proteins. This epoxide intermediate was not formed, however, by liver or lung microsomes. Of seven purified rabbit P450s known to be expressed in the olfactory mucosa, including 1A2, 2A10, 2A11, 2B4, 2E1, 2G1, and 3A6, P450s 2A10/11 were the most active, producing epoxy-DCBN and DCBN-protein adduct; P450 2E1 was the only other active isozyme. Interestingly, P450s immunochemically related to the rabbit 2As have previously been detected in rat, mouse, and human nasal mucosa. However, the identity of these 2A-related rodent and human nasal P450s has not been elucidated. In other studies, the expression of 2A3, 2A6, and 2A7, known members of the rat and human CYP2A subfamily with high homology to rabbit 2A10 and 2A11, was examined with use of RT-PCR. Preliminary results indicate that 2A3 is expressed in rat olfactory mucosa and both 2A6 and 2A7 are expressed in human nasal mucosa. The identification and characterization of CYP2A cytochromes in rodent and human nasal tissues may have important implications for risk assessment of environmental agents in the olfactory system.

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Cytochrome P450 from the olfactory organ of the spiny lobster: cloning, sequencing, and cellular localization.

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Each olfactory sensillum of the spiny lobster *Panulirus argus* contains the dendrites of several hundred chemosensory cells, as well as the processes of a number of auxiliary cells. Our prior studies demonstrated that sensilla have several biochemical activities that have the potential to participate in the clearing of odorants from the receptor environment. Among these is an enzymatic activity that can metabolize compounds that are substrates for the cytochrome P450 class of monooxygenase enzymes. We report here work aimed at the molecular characterization and cellular localization of olfactory cytochrome P450. Complementary DNA (cDNA) libraries were constructed in λ bacteriophage vectors from messenger RNA (mRNA) isolated from the lobster olfactory organ. The polymerase chain reaction (PCR) was used to screen this library for cDNA sequences encoding putative odorant-metabolizing enzymes. Selected products of PCR screening were then cloned, and their nucleotide sequences determined. We show here that the lobster olfactory organ cDNA libraries contain sequences similar to those from the lobster hepatopancreas that code for a cytochrome P450. Furthermore, we show by means of *in situ* hybridization that within the olfactory organ, these sequences are uniquely localized in the auxiliary cells.

Architecture and Connections of the Orbitofrontal Components of the Primate Olfactory Cortex

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The connections between the main olfactory bulb (MOB) and several primary olfactory cortices were analyzed in the marmoset monkey *Callithrix jacchus* by *in vivo* and *in vitro* anterograde and retrograde tracing techniques. Unilateral microinjections of WGA-HRP or bilateral applications of the fluorescent tracers Rhodamine and Fast blue into the left and right MOB, respectively, resulted in retrograde labeling of neurons in the laminae II and III of the trigonum olfactorium *(TRO*, anterior olfactory nucleus), the frontal component of the prepiriform cortex and the orbitofrontal area 13*A* of WALKER. Interhemispheric axonal collateralization, as revealed by double labeling, was restricted to the dorsal and lateral subdivisions of the TRO. The analysis of the anterograde tracing material showed that the TRO and the area 13*A* are the targets of MOB centripetal projections, while the medial component of the olfactory tubercle (*OT*) does not receive substantial input from the MOB.

In turn, multiple fluorescent tracer applications in both orbitofrontal and temporobasal components of the olfactory cortex (Fluorogold and Fast blue into the left and right MOB, respectively, Diamidino yellow into prorhinal area 28L, *proRC*, and Rhodamine into perirhinal area 35/36 of. Brodmann, *periRC*) resulted in dense retrograde labeling within laminae II and III of a circumscribed field within the transition area between the lateral TRO and the lateroposterior orbitofrontal (*LPOFC*) cortex, which receives input from the MOB (demonstrated by using Carbocyanide fluorescent tracing). Neurons grouped within the deeper part of LPOFC project to rostral sectors of the periRC, while those residing within basal parts are related to the MOB of both hemispheres; these arrangements suggest topographical organization of LPOFC-periRC and -proRC and TRO-MOB projection neurons. The present study indicates that the proisocortical LCOFC is involved in olfactory and limbic cortical circuitry and suggests the existence of separate channels connecting circumscribed fields of LPOFC to discrete subfields of the pro- and periRC and the primary olfactory relay center.

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EEG registration of Suprathreshold Odor Concentrations of Isoamyl Acetate and Androstenone: Application of Chaos Analyses

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Nineteen channels of EEG were recorded from 86 college students while they smelled pairs of bottles. One bottle per pair contained either isoamyl acetate (IAA) or androstenone (AND) (5-alpha-androst-16-en-3- one) dissolved in silicone. The other bottle contained silicone (the control). Subjects were prescreened for IAA and AND detection. EEG was collected during two two-second sniff periods per bottle for 8 trials at two concentrations, one suprathreshold and one subthreshold (subthreshold AND determined by AND osmic subjects). The order of odors, concentrations, experimental and control bottles, and hand, was counterbalanced within subjects. After each trial, subjects indicated which bottle contained the odor (detection), rated their confidence of detection and the odor's intensity. EEG was amplified using the NeuroSearch-24. EEG was sampled at 256 Hz. Approximate entropy (ApEn) analyses using the Pincus et al (1991) method for measuring regularity and complexity in time-series data were performed on 8 sec of EEG per trial, and odor minus control ApEn scores were displayed in topographic maps. Correct odor detections for the suprathreshold concentrations were IAA (99%) and AND (95% for osmics, 42% for anosmics). When subjects smelled suprathreshold IAA and AND, decreased complexity (decreased ApEn) was observed in anterior regions and increased complexity (increased ApEn) was observed in posterior regions. Habituation from sniff 1 to sniff 2 was observed as a decrease in relative complexity in posterior regions. The topographic pattern of ApEn scores was more complex for AND compared to IAA in both osmic and anosmic subjects. The ApEn statistic provides information that complements and extends traditional spectral analyses.

Pincus, S.M, Gladstone, I.M., and Ehrenkranz, R.A. (1991). A regularity statistic for medical data analysis. Journal of Clinical Monitoring, 7, 335-345.

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Magnetoencephalographically Identified Sources of Cortical Olfactory Activity in Man

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The orbitofrontal cortex plays an important role in the discrimination of olfactory stimuli (Zatorre and Jones-Gotman, 1991). Thus the aim of the present study was to investigate whether the primary neocortical projection areas are localized in the frontal or in the temporal lobe. Since magnetoencephalographical recordings seem to be more reliable in localizing neuronal generators of cortical activity than electroencephalographical techniques olfactory event-related magnetic fileds (OERMFs) were used in combination with magnetic resonance imaging.
Twelve healthy volunteers (5 male and 7 female subjects; 20 to 40 years) participated in the experiments. Stimulants (vanillin 2 ppm; hydrogen sulfide 2.5 ppm) were presented with a stimulus duration of 200 ms and an interstimulus interval of 20 s. The MEG was recorded with a 37 channel superconducting first order axial gradiometer (KRENIKON) in a magnetically shielded chamber. In order to define time relations between magnetic and electric components of the responses, olfactory event-related potentials (OERPs) were additionally recorded via an electrode (Cz versus A1 according to the international 10/20 classification) Control of eye blinks was conducted via the Fp2 lead (versus A1).
Data analysis indicated that the olfactory source of the OERMFs could be localized in the temporal lobe ipsilaterally to the stimulated nostril. Clusters of identified dipoles were located in the anterior and medial parts of the temporal lobe and close to the insula. Sources of the OERMFs could not be identified as easy compared to sources of the responses to the trigeminal stimulant CO_2. However, they were clearly discriminable from the somatosensory projection areas. These results are in accordance with earlier findings using maps of event-related potentials demonstrating two separate generators of olfactory and trigeminal responses (Hummel and Kobal 1992).

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Localization of Human Gustatory Cortex Using Functional Magnetic Imaging. J. HIRSCH[1,2], R. DeLaPAZ[1,2], N. RELKIN[2], J. VICTOR[2], L. BARTOSHUK[3], R. NORGREN[4], AND T.C. PRITCHARD[4] ([1]Memorial Sloan-Kettering Cancer Center, New York, NY, [2]Cornell University Medical College, New York, NY, [3]Yale University School of Medicine, New Haven, CT, and [4]The Pennsylvania State University, Hershey, PA)

We present the first direct observation of the location of human gustatory cortex using functional magnetic imaging (fMRI). Images were acquired on a General Electric Signa 1.5 Telsa scanner with echo-planar imaging using a T2*-weighted gradient echo sequence (TR 3000, TE 60, flip 30) with an in-plane resolution of 1.5 x 1.5 mm. Fourteen contiguous coronal slices (5 mm thick) extending anteriorly from the brain stem were imaged at 3 s intervals for 90 s during the baseline (resting) and stimulation (tap water, 1.0 M sodium chloride, and 1.0 M sucrose) conditions. The subjects were 2 normal volunteers, a right-handed male and an ambidextrous female. Approximately 8 ml of liquid were injected into the mouth (either by the subject or another person) at the onset of the stimulation period. Local cerebral hemodynamic changes, assumed to accompany neural activation, were evaluated with a multistage, single-voxel statistical analysis that compared signal intensities 1) between baseline and stimulation periods, 2) between tastant and water-control conditions, and 3) across multiple experimental runs (coincidence detection). Active regions were identified for both subjects in the insula and frontal operculum adjacent to the crest of the circular sulcus. Both subjects demonstrated multiple local regions of activity including areas activated by both tastants, areas activated by sucrose only, and areas activated by sodium chloride only. Most activity was located in the left hemisphere for both subjects suggesting that functional laterality may exist at the level of gustatory cortex.

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Evidence For A Relationship Between the Morphology and Response Properties of Gustatory Neurons in the Nucleus of the Solitary Tract.

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XUEGUO ZHANG (Henry Ford Hospital, Case Western Reserve University)
LAURA SCHWEITZER (University of Louisville School of Medicine)

In the present study we explored the hypothesis that the breadth of tuning exhibited by a given gustatory neuron is related to one or more of the neuron's morphological features. We used *in vivo* intracellular recording and labeling techniques to determine the morphology of individual neurons that responded to one or more the tastants in our stimulus array (distilled H_2O, 0.1 M NaCl, 0.1 M NH_4Cl, 0.1 M KCl, 0.01 N quinine HCl, 0.01 N HCl, 1.0 M sucrose, 0.1 M alanine, 1.0 M urea, 0.5 M NaCl, 0.1 N HCl). A total of 63 gustatory neurons were successfully labeled with Neurobiotin and reconstructed in three dimensions using the Eutectic Neuron Tracing System. We used two measures to examine the specificity of the gustatory neurons. In the first analysis, specificity was simply expressed as the number of tastants (1-11) that excited or inhibited a given neuron within 5 seconds of stimulus onset (correcting for spontaneous activity). Using this approach, we found that neurons that responded to only 1 or 2 stimuli had significantly more ($p < 0.05$) spines and a higher spine density than all other cells. There were a number of marginally significant ($0.10 \geq p \geq 0.05$) differences related to dendritic architecture as well, with stimulus-specific neurons exhibiting longer dendrites, more dendritic branching and fewer primary dendrites than the other gustatory cells. In our second analysis, we employed the breadth of tuning measure (H) described by Smith and Travers[1]. In addition to the number of stimuli eliciting a response, this measure also considers the strength of the response to each stimulus. We found that neurons with narrow breadth of tuning ($H < 0.2$) were significantly more widespread in the rostro-caudal dimension than all other neurons. There was also a trend for these cells to have a greater total dendritic length and larger somata. Our results suggest that the number and density of spines and the rostro-caudal extent of the dendritic tree are particularly important factors in establishing a given neuron's stimulus-specificity.

[1] Smith and Travers (1979) Chem. Senses and Flavor 4:215-229.
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Taste-induced c-fos expression in the rat hindbrain

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The distribution of the evoked expression of the immediate early gene c-fos was immunocytochemically visualized in the rat hindbrain after free ingestion of sucrose. Rats were trained to drink a 1 M sucrose solution for one week. Then, after a 22 hour food deprivation, the rats were allowed to drink the sucrose solution for 1.5 hour and were subsequently sacrificed. C-fos-like proteins within thin sections of the brain were immunostained by a conventional method. C-fos immunoreactive neurons were found within the nucleus of the solitary tract, parabrachial nucleus, dorsal motor nucleus of the vagus and nucleus ambiguus. These nuclei are related to sensory messages occurring during ingestive behavior such as general visceral information, information about taste quality and hedonic aspects of food and drink. The present study has proved that c-fos is a useful anatomical marker for activated neurons in the rat hindbrain during ingestive behavior.

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Nucleus S: A New Landmark for Taste in the Brain Stem.

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The taste representation in the solitary nucleus (SN) for the anterior tongue in hamsters was studied electrophysiologically with multi-unit recordings. Experiments involved 15 anesthetized hamsters *(Mesocricetus auratus)*. The SN taste area for each animal was mapped with the following stimuli: 0.1 M sucrose, 0.03 M NaCl and 0.1 M KCl. Then horseradish peroxidase (HRP) was iontophoretically delivered into the center of the taste area. Each animal at the conclusion of the experiment was perfused transcardially with 1% formaldehyde and 3.3 % glutaraldehyde. The brains were removed, cryoprotected, and frozen sectioned at 40 um. Five brains each were cut in the transverse, horizontal, and sagittal planes. All sections were processed with the Hanker-Yates method to visualize the HRP spot and taste maps were reconstructed for each plane. The method of reconstruction is based on the fact that the coordinates of the HRP spot and the recording sites are known. However, we needed a consistent histological landmark in the tissue in order to compare recording sites in different brains. A small cluster of cells darkly stained for Nissl substance with cresyl violet can be observed outside the taste area near the SN rostral pole. This cluster is restricted to 2-3 sections. Cell bodies, elongate in transverse and sagittal, but nearly round in horizontal sections, are medium-sized (about 10 x 20 x 20 um) and appear tightly grouped together. This cell cluster has not been previously identified and was named Nucleus S. Nucleus S is, on average, 430 um rostral, 110 um ventral, and 60 um lateral to the center of the SN taste area. These distances were established as follows. A positional centroid of taste was calculated for each case based on recorded coordinates and its relationship to the HRP spot calculated. Then the distance was measured histologically between the HRP spot and Nucleus S. Finally the distance between Nucleus S and the centroid was calculated. Nucleus S is very close to SN and recognizable in all three planes, which makes it a superior landmark for taste in the brain stem.

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Recognition of Deficient Nutrient Intake Scanned by a Functional MRI in the Brain of Rat with L-lysine Deficiency .

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Each L-amino acid (AA) in plasma and brain remains unchanged all day long while normal diet is available. Once L-lysine (Lys) deficient diet was offered to rats, Lys in plasma and brain declined. When solutions of AAs were offered, they selected the Lys solution and their food intake and growth normalized. The single neuron activity in the lateral hypothalamic area of these rats suggested that neural plasticity occurred, specifically responding to Lys, both iontophoretic application and during ingestion of AA. The recognition site for deficient nutrient intake in the brain of rat with Lys deficiency was identified by non-invasive magnetic resonance imaging (MRI) developed to monitor changes in cerebral blood flow and oxygenation in rats. Related MRI signal intensity changes in T2* weighted images of the brain of rats with Lys deficiency were studied using a MR Imager (4.7 tesla, 40 cm bore in diameter). Wistar strain male rats (6 weeks of age, N=6, in each group) fed with Lys deficient diet for 4 days, were adapted to settle in the center of the bore. When they received a Lys injection intraperitoneally (0.2 M, 10 ml/kg) higher signals in the medial and lateral hypothalamus appeared in T2* weighted images. This higher signal in these areas caused by the Lys treatment reflecting the increased oxygen consumption of neurons lasted for 30 min, and then gradually decreased. These changes never occurred in any other areas of the brain of rats with Lys deficiency, ie. thalamus, cortex, hippocampus, etc. There were no changes in the signal with injection of other AA or the saline control. In addition, oxygen consumption in the brain of rats without Lys deficiency was not altered by intraperitoneal Lys injection. Previously reported studies (Mori M, Kawada T, Ono T ,Torii K. Physiol.Behav. 49:987-995; 1991, Tabuchi E, Ono T, Nishijo H, Torii K. Physiol.Behav. 49:951-964; 1991) indicate neural plasticity in the CNS to Lys, subsequent to its ingestion in Lys deficient animals. The present results suggest that in essential AA deficiency, the medial and lateral hypothalamus may play important roles in recognition responses to particular deficient nutrients.

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The Influence of Conditioned Preferences in the Rat Nucleus Tractus Solitarius: Only a Matter of Time.

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Conditioned taste preferences (CTP's) may be created by pairing a novel taste (CS) with the intragastric infusion of nutrients (US). We recorded taste-evoked activity in the NTS of rats with CTP's to either 0.1 M $MgCl_2$ (Mg^+) or to 0.01 M citric acid (Ci^+) to determine whether the afferent code was modified by this conditioning experience. Forty-seven rats were trained in Brooklyn and transported to Delaware for electrophysiological recording. Behavioral tests were conducted immediately before recording to exclude any rat that did not show >75% preference for the CS^+ over both the CS^- and water. The two neural data sets were composed of single unit responses from 15 Mg^+ (N=6l) and 12 Ci^+ (N=68) rats. Three neural subgroups--oriented toward NaCl, HCl and glucose, respectively--were identified by cluster analysis in each data set. There was no significant change in mean evoked activity to either CS^+, either across all neurons or within any subgroup. A taste space was generated from each data set: the positions of $MgCl_2$ and citric acid were unaltered by the conditioning procedure. Therefore, the gustatory neural code across neurons was not affected by development of a CTP. Temporal aspects of the evoked activity were then examined. The phasic burst that characterizes responses to non-sweet stimuli was suppressed to citric acid in Ci^+ rats. Thus, the temporal profile was transformed to be less like that of HCl ($p<0.01$) and more like that of glucose ($p<0.01$), with the result in the temporal taste space that citric acid abandoned the sour bitter stimuli and joined the sugars. The time course of activity to $MgCl_2$, however, was unmodified in Mg^+ rats. A phasic burst of activity is **generated** as the temporal signature of a stimulus to which an **aversion** has been created (Chang & Scott, 1984). Here we see the converse: the phasic response is selectively **reduced** for a stimulus to which a **preference** has been conditioned. The effect was limited to citric acid, which occurs in the natural environment of rats and has been associated throughout evolution with health consequences.

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Effect of solvent and rinsing in human taste sensitivity to PTC and NaCl.

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The solvent chosen for gustatory psychophysics is generally distilled water, which has a taste. An alternative is to use a low concentration NaCl solution, to which subjects are adapted, so that it is tasteless.Subjects were required to discriminate between solvent "blanks"and low concentration NaCl in one condition and PTC in another. NaCl discrimination tended to be better using distilled water as a solvent while PTC discrimination was better using adapted NaCl as the solvent. Interstimulus solvent rinses tended to improve discrimination. Results were interpreted in terms of adaptation, sensory disparity and after-effects.

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Perception of, and preference for sweetness in foods by Japanese and Australians.

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Little is known about the preferred level of sweetness for foods that are common to two cultures and whether or not preference differences are determined by differences in intensity perception. We took three foods common to Australia and Japan (breakfast cereal, orange juice and vanilla icecream), varied the sucrose in each across four levels, and asked panels of adults in each country to rate sweetness intensity and preference for the resulting twelve products. With the exception of the lowest sweetness level in icecream, Japanese and Australians agreed on perceived intensity of all the products. However, there was no similar agreement for preference: differences between the two groups were found in each food type. In breakfast cereals, preferences tended to increase with increasing sugar content in both groups, but the cultures diverged at a sugar content 25% higher than the common standard. For orange juice, the Japanese preference function was flat whereas Australians showed a decline at high sucrose levels. For icecream, Japanese showed higher preference than Australians for the three highest sweetness levels. The results of this study support our earlier finding with sweetness in common foods, that both groups rate intensity identically. However, by contrast with preference for simple solutions, preferences were not identical in the two groups and differed significantly despite equal intensity ratings. In the two cultures, variation in preferences for sweetness is not determined by differences in intensity judgement. Factors other than ability to perceive taste intensity, such as interactions with other sensory attributes, or culture-bound expectations of what a particular food should taste like, possibly determine sweetness preference in foods common to different cultures.

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A Heuristic Model of Sensory Adaptation.

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CAREY D. BALABAN (University of Pittsburgh)

Sensory adaptation is commonly defined as a reduction in the response of a system to a steady input. This view, however, overlooks the principal function of adaptation, which is to permit an organism to respond to changes in stimulus intensity over a wide dynamic range. A simple mathematical model is presented that demonstrates a number of properties of sensory systems, including: temporal summation, transient response, response decrement, range shifting, Weber's Law, and phase leading during cyclical stimulation. This model, based on simple interactions between rapidly and slowly adapting sensory signals, predicts the results of Gent (1979) Kelling and Halpern (1983), McBurney (1976), and Smith and Bealer (1974). We argue that adaptation should be considered at this functional level as a fundamental property of sensory systems. The detailed structure and parameters of the model will vary with the functional requirements of the particular sensory system.

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Strong Acids Are Indiscriminable at Equal pH. PAUL A.S. BRESLIN and GARY K. BEAUCHAMP (Monell Chemical Senses Center).

As part of a research program designed to investigate the coding dimensionality of taste (presumedly related to the number of receptor classes) we have initiated studies to determine if stimuli falling into a common class (e.g., sweet; sour) are perceived as identical using forced choice methodology. We previously reported that moderate concentrations of three sugars, fructose, sucrose and glucose are indiscriminable when their concentrations are adjusted appropriately (*Chem. Sens.*, 17: 599. 1992). We have now conducted similar experiments to see if matches could be obtained between three strong acids: hydrochloric (HCl), sulphuric (H_2SO_4), and nitric (HNO_3). The experiments consisted of a series of two alternative forced-choice (duo-trio) trials comprised of three sequential sip & spit exposures in which the subjects had to state whether the 1st or 3rd stimulus was different from the 2nd. Across sets of trials, the concentration of one acid was held constant while the concentration of the other acid was varied semi-randomly. The order of the solutions within the three cups was counter-balanced across trials. For all subjects (n=3), we found characteristic concentrations of the test-acids HNO_3 (0.005M) and H_2SO_4 (0.0025M) that were indiscriminable from the standard, 0.005M HCl; test-acid concentrations that were higher or lower than the match point were discriminable following an inverted bell-shape function. Next, subjects were asked to discriminate between the two acids after the concentration of HCl standard was increased. The subjects failed to discriminate for a concentrations of the test acids that were scaled up an appropriate amount, and the match between these compounds was maintained at a fixed concentration ratio. These acids were indiscriminable at the same pH values. Thus the protons were the only aspect of the stimuli that contributed to their taste quality and intensity. We surmise that the tastes elicited by these acids are indistinguishable for a specific concentration ratio because they act identically upon the relevant receptor mechanisms and give rise to indistinguishable neural signals.

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Qualitative Evaluation of Taste Stimuli by Cross-Modality Matching with a Color System

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LISA ROSS (The Pennsylvania State University)
ANGELITA MARIN DE GUTIERREZ (The Pennsylvania State University)

The purpose of this research was (1) to explore the use of colors to describe the quality of taste stimuli as an alternative to semantics and (2) to use that cross-modality matching technique to assess qualitative differences among sweet stimuli and among bitter stimuli. Twenty-seven equi-intense stimuli were described by 10 judges using the Munsell book of colors. An initial set of 9 stimuli including a representative of each basic taste was used by the subjects to build their own spectrum of colors to match taste qualities. Subjects were asked to pick one or several color(s) to describe the taste quality(ies) of each solution. All subjects were able to develop a system of colors to describe the quality of the stimuli, even though they did not use the same colors. For each subject, the stimuli were plotted in a three-dimensional space of taste qualities corresponding to the color(s) used to describe them. Results indicate that sweetness and bitterness are not unitary, and that semantics are not sufficient to describe taste quality.

Dietary Fat Intake Alters The Selection Of Discretionary Fat.
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Human subjects adhering to low fat diets during clinical and laboratory studies alter their preference for regular and fat-modified food products. A study was designed to evaluate whether the level of fat in subjects' self-selected diets would affect their hedonic ratings and selection of commercially available regular and fat-modified foods. Subjects were recruited by advertisement. Dietary intakes were determined by interviews and diet records. Low fat consumers obtained 25.6% of their daily caloric intake from fat and the high fat group consumed 37.8% of their calories as fat. Subjects completed hedonic and preference questionnaires for regular and fat-modified table spread, salad dressings and cream cheese during taste tests. Preference ratings for each food item were determined by asking the subject to rate "how much they liked the food" and "how likely they were to eat the food item again". The preference ratings given by low fat consumers did not differ from those of high fat consumers. Following completion of taste testing, the subjects were asked to prepare a food item using the product as a source of discretionary fat. Low fat consumers consistently selected smaller quantities of both regular and fat-modified foods compared to high fat consumers. The amounts selected were independent of the level of fat contained in the products. Neither group was able to differentiate between the varying levels of fat present in the foods. The amounts selected were not altered in either group by providing the subjects with information pertaining to the fat content of the foods. In response to the Health Concerns Questionnaire, low fat consumers rated the item evaluating their concern about dietary fat significantly higher than high fat consumers. The preference ratings for regular and modified fat food products do not differ between subjects who self-select low and high fat diets. However, low fat consumers consistently select smaller quantities of both regular and fat-modified food products than high fat consumers suggesting that cognitive restraint plays a role in altering food selection patterns.

Gustatory Function After Oral Maxillofacial Surgery. A. MOTT+, D. SHAFER*, D. MILLER*, D. SANGER*, M. BANKI*, L. NORTON*. (Univ. of CT Health Center, Farmington, CT *School of Dental Med., +School of Med.).

A potential complication of orthognathic surgery (OS) or third molar extraction (TME) is damage to peripheral nerves carrying taste information (chorda tympani via the lingual and greater superficial petrosal via the palatine nerves). The purpose of this study is to determine the frequency, type, location and severity of taste loss and the time course and pattern (whole mouth and spatial quality; spatial loci) of taste recovery after these procedures. Subjects recruited from the Univ. of CT Oral Maxillofacial (OMF) surgery clinic were prospectively studied, with baseline pre-op tests serving as internal controls. Gustation was measured by suprathreshold whole mouth and spatial (ant/post tongue, palate) tests of NaCl (salty), citric acid (sour), sucrose (sweet), and quinine HCl (bitter) taste. The first six TME subjects (3 females, 3 males; mean age = 22 [range 15-36]) were tested one mo. after surgery. Five had all third molars removed; one had mandibular extractions only. Whole mouth scores were lower than baseline at one mo. post-op (2-tailed paired t-test) for NaCl ($p=.002$), sucrose (.03), quinine HCl (.03) and overall taste ($p=.006$). Citric acid scores showed a trend ($p=.06$) toward decreasing sensation. Spatial testing showed a similar frequency of localized ageusia when compared to baseline. Spatial intensity ratings, however, were lower for all taste qualities (each $p\leq.0001$). In the ant. tongue, NaCL taste was preserved, and sucrose, citric acid, and quinine HCl tastes were decreased. In the post. tongue, only sucrose taste was diminished. On the palate, all taste qualities were affected except sour. Data are also available for the first 5 OS subjects (4 female, 1 male; mean age = 23 [range 18-36]). Four had both maxillary and mandibular procedures, one had mandibular only. Post-op whole mouth scores at 2 & 6 mo. did not differ from baseline. Spatial testing 2 mos. post-op showed an increase in the number of localized ageusic areas from baseline (Fisher's exact test) for NaCl ($p=.04$) and citric acid ($p=.006$), with a trend for sucrose ($p=.07$). Some reversal of localized ageusia was noted by 6 mos after surgery. Spatial intensity ratings 2 mos. post-op were lower than baseline (2-tailed paired t-tests) for NaCL ($p=.04$) and quinine HCL ($p=.02$). Six mo. post-op scores remained lower than baseline for NaCL ($p=.05$) and quinine HCL ($p=.0001$), with a trend for lower quinine HCL scores at 6 mos. compared to 2 mos. ($p=.08$). None of the subjects reported post-op. subjective taste alterations. We conclude that decreases in measured whole mouth and spatial gustatory function can occur after OMF procedures. Within each subject group, differential taste quality effects were noted and suggest varying susceptibility to damage or rate of recovery within specific taste fields. Localized ageusia may be more likely after OS than TME. Whole mouth taste was preserved 2 mo. after OS despite measurable spatial deficits.

This study was funded partially by NIDCD, PH50-DC00168.

Epidemic Phantogeusia.
ALAN R. HIRSCH (Smell & Taste Treatment and Research Foundation)
MARK A. DREXLER (University of Illinois Medical School, Chicago)

While phantogeusia has been reported to occur in approximately 5% of the normal population, epidemic phantogeusia remains undescribed. Metalic phantogeusia afflicted 8 co-workers (6 female, 2 male), whose ages ranged from 30 to 56 years. All experienced phantogeusia within a 6 month period while working at a chemical production plant. The phantogeusia lasted anywhere from minutes to 12 hours, with an average of 2 to 3 hours, except for proband which has persisted more than 9 months. Onset of symptoms was within minutes to hours of entering a specific room in the plant. All but one had dental work in the past. Half had allergies to pollen, 3 of 8 were smokers. All drank 1 to 4 beer per month. All but the proband had resolution of the phantogeusia on leaving the described room. Indoor air quality survey testing of the plant in multiple locations revealed 60% mixed air from the office and neighboring laboratories at the site of precipitation of phantogeusia. At that site two samples of total hydrocarbons exceeded acceptable limits. Postulated origins for the persistent phantogeusia in the proband included: pseudophantogeusia, abnormal salivary composition, infectious processes, metabolic and hereditary disorders, neurologic disease, psychiatric illness, release of heavy metal from fillings, persistent VOC contamination, mass illness, or secondary generalization.

Response Properties of Crayfish Local Deutocerebral Interneurons following Stimulation of the Olfactory Pathway by Odorants.
DE FOREST MELLON, JR. (University of Virginia)

I have used intracellular electrodes to obtain records of electrical activity from local interneurons in the crayfish deutocerebrum, while stimulating the olfactory pathway with odorant solutions. Crayfish were decapitated, the heads were pinned out in a lucite chamber, and the lateral antennular filaments were inserted into a plastic tube that passed through one wall of the chamber. The antennules in the tube were isolated from the chamber with a vaseline seal at their basal segments. The medial artery and one of the paired lateral cephalic arteries in the head were cannulated and supplied with chilled, oxygenated crayfish saline. An olfactometer delivered either dechlorinated tap water or temporally-controlled slugs of odorant solution to the antennular filaments within the plastic housing. Local interneuron somata, located in cell cluster # 11, were penetrated with potassium acetate-containing microelectrodes. 0.02% Tetramin solution or, alternatively, a mixture of five amino acids (10^{-5}M) applied to the antennules generated either excitatory or inhibitory responses in different local interneurons. The response of some cells to odorant stimulation consisted of both an excitatory and an inhibitory component, with excitation being reduced as the duration (=intensity) of the stimulus pulse was increased. In these neurons, prolonged stimulation generated a brisk "on" response, followed by diminishing inhibition, and stimulus cessation was often accompanied by an "off" response. Staining of the local interneurons with biocytin revealed extensive dendritic arborization within the ipsilateral olfactory lobe, involving most or all glomeruli. The major neurites from a cell distributed themselves around the periphery of the olfactory lobe before subdividing and then penetrating individual glomeruli. Axonal outputs from the impaled neurons were never clearly identified, but in some cells there was additional arborization in both the lateral antennular neuropile and the olfactory-globular tract neuropile.

Differential Effects of βAP Application Suggests the Olfactory Cortex is Inherently Susceptible to Alzheimer's Disease. J.A. LONDON, L. NIEGO, T.S. DONTA (UCONN Health Center, Farmington, CT)

In Alzheimer's disease (AD) pathologies are manifested first and most strongly in the frontal, temporal and parietal lobes, while the brain stem or the somatosensory cortex are almost completely spared. One hypothesis suggests there is variable access of brain regions to ß-amyloid peptide (βAP), the more access, the more the region is affected. A second hypothesis suggests there is some inherent property of a subpopulation of cells making them more susceptible to the affects of βAP. If the first hypothesis were true, all regions would be equally susceptible to the effects of βAP. If the second hypothesis were true, exposure to βAP would result in only certain regions being affected. Using organotypic cultures, we have begun to test these hypotheses. Organotypic cultures, 200 μ thick sections of rat pup brain cut in the transverse plane, consist of one hemisphere containing several different cortical areas. Cultures retain their structural and electrophysiological integrity for many months. Cultures containing different brain regions were exposed to the 25-35 fragment of βAP and examined at 2, 4, 6, 8, 12 and 24 hours after exposure. Control cultures consisting of non-exposed cultures and cultures exposed to vehicle alone were taken at the same time points. The ratio of dead to live cell was determined by comparing the number of cells stained with ethidium bromide (stains the nucleus of dead cells) to the number of cells stained with calcein (stains the cytoplasm of live cells). After counting, cultures were fixed and Nissl stained to determine which structures were most affected. Preliminary evidence suggests the olfactory cortex exhibits the effects of βAP first, followed by the entorhinal cortex. The olfactory cortex lost approximately 50% of its cells in 4 hours. The entorhinal cortex lost the majority of its cells at 6 hours. The somatosensory and somatomotor cortex, as well as the brain stem were relatively unaffected, gradually losing cells over the 24 hour period. These data suggest there is an inherent difference in brain structures that cause them to be differentially affected by βAP.

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Ultrastructural study of serotonergic innervation of olfactory glomeruli in adult rats. WEILIN LIU and MICHAEL T. SHIPLEY (*University of Cincinnati*)

Serotonergic afferents to the main olfactory bulb arise from the dorsal and median raphe nuclei to the olfactory bulb. Using retrograde and anterograde tracing methods and immunocytochemistry for serotonin (5-HT), we previously demonstrated that the 5-HT fibers are more densely distributed in the glomeruli than in the infraglomerular layers of the olfactory bulb (McLean and Shipley, J. Neurosci. 7:3016). We suggested that the most likely targets of raphe nucleus serotonergic synapses are either the dendrites of mitral/tufted cells, periglomerular cells or possibly the primary olfactory neuron terminals themselves. The present study used EM-immunocytochemistry (EM-ICC) to investigate the postsynaptic elements of the serotonergic fibers in the glomeruli of the olfactory bulb. Adult rats (250-350 g) were perfused with 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer, and processed for 5-HT EM-ICC. Serotonergic immunoreactive terminals were easily recognized by the presence of the dark DAB reaction product. Serotonergic immunoreactive terminals formed asymmetrical synapses with dendritic profiles which differed in size, shape and cytoplasmic content. Dendritic profiles with a regular outline, clear cytoplasm and regularly arranged array of microtubules were similar to previous descriptions of mitral/tufted cell dendrites. Dendritic profiles with irregular outlines and a cytoplasm containing scattered agranular and a few granular vesicles were probably the periglomerular cell dendrites. Serotonergic immunoreactive terminals had synapses with both types of dendrites. A few 5-HT synapses were identified on the initial part of the dendrites of the periglomerular cells. Of 20 synapses observed, none were between 5-HT immunoreactive terminals and the primary olfactory axons in the glomeruli of the olfactory bulb. The functional significance of raphe nucleus 5-HT projections to the glomeruli of the olfactory bulb is unknown. A potential role for 5-HT synapses in olfactory glomeruli might be to mediate dishabituation to behaviorally salient olfactory stimuli as in aplysia. Alternatively, serotonin release in rat somatosensory cortex depresses sensory-evoked neuronal discharges relative to background firing, which might decrease the sensory signal-to-noise ratio. Serotonin also seems to depress the actions of other neurotransmitters on neocortical neurons. Neurophysiological studies of raphe stimulation and 5-HT application in the olfactory glomerular layer are needed to assess potentially analogous function of 5-HT in the olfactory bulb.

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Connections of the Olfactory Bulb in Chinook Salmon. STUART P. MATZ (Institute of Neuroscience, U of Oregon) GREG T. HOFELDT and TERRY T. TAKAHASHI (Institute of Neuroscience, U of Oregon. Eugene, OR 97403)

The connections of the olfactory bulb were studied in juvenile chinook salmon (*Oncorhynchus tshawytscha*) using the retrograde and anterograde transport of biotinylated dextran amine (BDA). The use of BDA allowed us to clearly distinguish axon terminal endings from fibers of passage. Terminal-like boutons were present in the ventral (Vv), lateral (Vl) and supracommissural (Vs) nuclei of the ventral telencephalon and in the lateral (Dl) and posterior (Dp) zones of the dorsal telencephalon. Terminal fields were also present in the preoptic area and in posterior tuberal region of the ventral diencephalon. A projection to the contralateral bulb is also observed.

BDA retrogradely labels neurons in a golgi-like manner allowing one to visualize the morphology of olfactory bulb efferents. Three types of neurons in the bulb were retrogradely filled by injections of BDA into the posterior zone of the dorsal telencephalon. Retrogradely labeled neurons include mitral cells in the external cellular layer, ruffed cells in the external cellular layer and neurons in the internal cellular layer. Although it had been known that both mitral cells and neurons in the internal cellular layer project out of the bulb, the use of BDA allows for an accurate description of the morphology of these extrinsically projecting neurons. This is the first evidence that ruffed cells of the olfactory bulb have extrinsic projections.

This work was supported by NIH Systems Physiology Training Program (GM07257).

Ultrastructural Characterization of Tyrosine Hydroxylase and GABA Immunoreactive Processes in Rat Olfactory Bulb Glomeruli. JUAN C. BARTOLOMEI and CHARLES A. GREER (Sections of Neurosurgery & Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510).

It is now well accepted that the rat olfactory bulb glomerulus (GLOM) contains subpopulations of periglomerular (PG) cells immunoreactive for several different neurotransmitters/modulators. Prior studies from our laboratory described the synaptic organization of tyrosine hydroxylase immunoreactive (TH-IR) processes in rat olfactory bulb GLOM. It is not yet clear, however, if the synaptic organization of TH-IR PG cells is representative of the other subpopulations of PG cells also contributing processes to GLOMs. To address this question we are comparing the synaptic organization of TH-IR PG cells and PG cells immunoreactive for GABA (GABA-IR) in rat olfactory GLOM. Adult rats, 200-300g, were anesthetized and perfused with 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1M phosphate buffer. Tissue sections were incubated for 48 hrs. in primary TH Ab and GABA Ab (Eugene Tech Int., 1:1200 and 1:1000 respectively) and then processed for routine immunoelectron microscopy. Qualitatively, both TH-IR and GABA-IR cells appeared similar. IR cell bodies in the glomerular layer were similar to the PG cells described by Pinching and Powell (Cell Sci., 1971, 9:305). Characteristics included a thin rim of cytoplasm surrounding an invaginated nucleus and asymmetrical synapses onto the cell body. The olfactory nerve terminals (ONs) were clearly identified based on their electron dense axoplasm. The ONs established asymmetrical synapses onto IR and non-IR processes. Several large electron lucent processes, presumably mitral/tufted (M/T) cell dendrites, were seen making asymmetrical synapses onto IR and non-IR processes. Both IR and non-IR processes made symmetrical synapses onto M/T dendrites. There were several instances of reciprocal dendrodendritic synapses involving IR and non-IR processes but none involving two IR processes. Quantitatively, the TH-IR processes received a higher density of synapses from ONs and made more symmetrical synapses onto M/T cell dendrites than the GABA-IR processes. The GABA-IR processes received a higher density of asymmetrical synapses from M/T cell processes than did the TH-IR processes. The M/T cell processes received the highest density of axodendritic synapses. These results support the notion that subpopulations of PG cells are heterogeneous in their synaptic organization.

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Topological Distribution of Olfactory Receptor Cell Axons in Olfactory Bulb Glomeruli: A Confocal Microscope Analysis of DiI Staining.

JEFFREY M. DEMBNER and CHARLES A. GREER (Sections of Neurosurgery & Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510).

Organization of the olfactory bulb glomerulus is not well understood. It is recognized that glomeruli are an initial site of synaptic interactions including both primary afferent axodendritic synapses and local circuit dendrodendritic synapses. However the degree to which these interactions are homogeneously distributed throughout the glomerulus, or the degree to which compartmentalization may occur, is not known. We previously demonstrated with Golgi preparations that single olfactory receptor cell axons occupy very restricted regions within the glomerular neuropil (Halasz & Greer, 1993). In the current report, we extend these findings by examining the distribution of DiI stained fascicles and single axons as they approach and penetrate glomeruli. Two-week old Sprague-Dawley rat olfactory bulbs were immersion fixed in 4% paraformaldehyde prior to placement of DiI crystals into the olfactory nerve layer. Dye transport took place at room temperature and averaged 3 weeks in duration. The analyses were conducted by capturing thin optical images with a BioRad 600 Confocal Laser in order to understand the three-dimensional distribution of labeled processes. Single axons were easily resolved and exhibited both terminal as well as *en passant* varicosities. The number of intraglomerular axonal collaterals ranged from 2 to 6, consistent with our observations in the Golgi stained tissue. In transverse plane olfactory bulb sections, the terminal field of single axons appeared to distribute in a cylindrical fashion. While the x- and y-axes, the width and height of the cylinder, were quite variable, the rostral-caudal z-axis consistently ranged from 10 to 12μm. Most significantly, many small fascicles appeared to enter glomeruli in a compartmental manner in two respects. First, fascicles did not arborize homogeneously throughout the glomerulus, suggesting that any single fascicle occupies a restricted region. Second, fascicles tended to occupy the outermost portion, or shell, of the glomerulus while the innermost portion, or core, remained largely free of stained processes. These data support the hypothesis of a subcompartmental organization of primary olfactory afferent subsets within the glomerulus. Further, they suggest that primary afferent synapses may occur predominately within the shell of the glomerulus.

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Is there a difference in somatic granule cell spines between male ferrets at different sexual states?

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The ferret is a seasonal breeding mammal. Behavior, testosterone level and even brain weight change during the year. In spring/early summer plasma testosterone concentration and brain weight are high; sexual behavior is exhibited. In fall/winter these values are low and no sexual behavior can be observed. In this species the olfactory bulb (BO) is well developed and constitutes a remarkable part of the brain. This fact together with behavioral data indicates that olfaction is the dominant sensory system when searching for prey and for mate recognition. Surprisingly, there is no well developed accessory olfactory bulb (responsible for social odors in many mammals) in this species. Therefore, it is reasonable to assume that the main olfactory system has to take over this function. For odor information processing, interneurons like the granule cells in the BO are responsible. It therefore seemed justified to investigate whether granule cells do show seasonal differences. Granule cells bear somatic spines. Golgi impregnation studies revealed no difference in the percentage of somata bearing spines during the course of the year. Even the density of spines per soma remained unchanged in males at different hormonal states. Therefore, one has to conclude that the number of somatic spines is independent from seasonal and hormonal influences and that somatic spines do not play an obvious role in sexual odor information processing.

Olfaction in Rats Treated with 400 mg/kg 3-Methylindole

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Anterograde transport of HRP from olfactory epithelium to olfactory bulb glomeruli is severely disrupted in rats after treatment with 350 mg/kg of 3-Methylindole (3-MI; Setzer and Slotnick, AChemS, 1992). In Experiment 1, 6 rats were trained to detect 0.15% vapor saturation of isoamyl acetate, treated with 400 mg/kg of 3-MI, tested for retention beginning on post-treatment day 4 and, immediately after achieving criterion performance, treated with WGA*HRP to assess anterograde transport from the epithelium. Two rats proved anosmic in 12 test days. The other 4 performed initially at chance, but reached or acceeded criterion of 85% correct responding after 3-6 test days. These rats had fewer than 1% of their glomeruli filled with dense (control level) anterograde transport and fewer than 10% (range: 4.6% - 9%) of glomeruli had any detectable anterograde transport. The correlation between percent of glomeruli with dense reaction product and trials to criterion was 0.93. Glomeruli with dense reaction product were located primarily in the ventromedial aspect of the bulb and there was essentially no detectable transport in the dorsal aspect of the rostral half of the bulb. Controls and treated rats trained on a visual detection problem had no post-treatment deficits. In Experiment 2, rats were trained to detect 0.03% concentrations of isoamyl acetate, peppermint extract, proprionic acid and ethyl acetoacetate and tested on 0.03%, 0.003%, 0.0003% odor concentrations after treatment with 400 mg/kg of 3-MI. Four of 7 rats were anosmic for each of these odors, but the remaining rats could detect most or all concentrations of each test odor although they made more errors than did controls. These rats had detectable reaction product in only about 12% of their glomeruli while the anosmic rats had less than 6%. Glomerular regions associated with 2-DG uptake for peppermint and proprionic acid (as revealed in prior studies) had little or no reaction product.

NIDCD R01 DC01266

Anatomical and Electrophysiological Identification of Neurons in the Caudal Layers of the Salamander Olfactory Bulb.

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The salamander olfactory bulb contains a large number of putative dopaminergic neurons that innervate the glomeruli (K.A. Hamilton and S.S. Foster, *Neurosci. Abstr.* 17:637, 1991). Most of the dopaminergic cell bodies occur near the junction of the granule and mitral cell layers and in the caudal granule cell layer near the ventricle. In the present study, intracellular recording and staining methods were used to identify cells in the caudal layers of the salamander bulb that exhibit morphological characteristics of putative dopaminergic cells that innervate the glomeruli.

Recordings were obtained with fine-tipped microelectrodes from 32 cells in isolated hemibrain preparations, and 16 of the cells were stained with Neurobiotin. Seven of the stained cells exhibited morphological characteristics of mitral and/or tufted cells, and responses of the cells to olfactory nerve stimulation resembled responses of salamander mitral/tufted cells that have previously been recorded *in vivo* (K.A. Hamilton and J.S. Kauer, *J. Neurophysiol.* 59:1736, 1988). Four of the stained cells exhibited morphological characteristics of granule cells, and responses of the cells to olfactory nerve stimulation resembled responses of salamander granule cells that have previously been recorded *in vitro* (D.P. Wellis and J.S. Kauer, submitted). The remaining five cells could not be positively identified using morphological and electrophysiological criteria. The cell bodies of these cells were located in the caudal granule cell layer.

Both the mitral/tufted cells and cells in the caudal granule cell layer of the salamander bulb exhibited morphological characteristics of putative dopaminergic cells that innervate the glomeruli. In ongoing studies to determine if mitral/tufted cells or cells in the caudal granule cell layer could be dopaminergic, olfactory bulbs containing dye-injected cells will be processed to reveal both the injected cells and immunoreactivity for tyrosine hydroxylase.

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Behavioral-Genetic Studies of Olfactory Perception.
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Individual differences in olfactory sensitivity are frequently examined with reference to gender, age and experiential events. Twin studies of olfaction can advance current research in this area, as they may identify genetic factors underlying observed interindividual variation. An adolescent and adult twin sample, composed of 46 monozygotic (MZ) and 37 dizygotic (DZ) twin pairs, completed measures to assess different aspects of olfactory sensitivity. The mean ages were 29.38 years (SD = 18.14) for MZ twins and 21.90 years (SD = 9.59) for DZ twins. Findings from the University of Pennsylvania Smell Identification Test (UPSIT) and a PEA detection threshold test are reported. Age- and sex-corrected intraclass correlations suggested a genetic influence on these measures for males, but not for females. Females scored significantly higher than males on the UPSIT, while gender differences on the PEA test were not observed. Twins also rated UPSIT items on scales of Intensity, Pleasantness, Irritation, Familiarity and Warmth. Genetic effects were observed for judgments of Intensity for selected classes of items. The effects of age, smoking, olfactory and medical ailments, and timing of the menstrual cycle on olfactory perception were also examined. It may be that genetically influenced mechanisms on performance include genetic effects on degeneration of olfactory neuroepithelium and/or predisposition to viral and infammatory disease, which are known to affect olfactory functioning. Preliminary findings from new ongoing twin studies of olfactory perception (e.g., study of kin recognition, using t-shirts worn by MZ and DZ twins; twin analyses of genetic influences and gender differences in specific anosmias, using adult MZ and DZ twins and young opposite-sex twins [test kits provided by Olfacto-Labs]; and a study of genetic influences on the UPSIT, using MZ and DZ twins reared apart) will be summarized as available.

These projects were supported by the Olfactory Research Fund and CSU Fullerton (Dr. Nancy L. Segal) and NIDCD, PO1DC00161 (Dr. Richard L. Doty).

Discrimination of Odortypes Determined by the Major Histocompatibility Complex Among Outbred Mice. KUNIO YAMAZAKI[1], GARY K. BEAUCHAMP[1], JUDITH BARD[2] & EDWARD A. BOYSE[2] ([1]Monell Chemical Senses Center, Phila., PA; & [2]Univ. of Arizona, Tucson, AZ).

Genetically determined body odors that distinguish one mouse from another are termed odortypes. The best known odortypes, highly expressed in urine, are those specified by H-2, the major histocompatibility complex of the mouse, but other odortypes originate from unidentified loci in the rest of the genome, including both sex chromosomes. The definition of H-2 odortypes, and evidence that their perception affects reproductive behavior, has so far depended mainly on studies with inbred mouse strains whose genetic differences are confined to the H-2 complex of genes. The purpose of this study is to determine whether free segregation of the genome as a whole would substantially affect the perceptibility of H-2 odortypes. To simulate fetal conditions more closely, a freely segregating population was bred from crosses involving four unrelated inbred strains contributing four different H-2 haplotypes. After H-2 typing, this outbred population was divided into four groups of freely segregating mice, comprising the four distinct genotypes represented, to serve as donors of urine for evaluation in the standard Y-maze system used in the training and testing. With respect to utility in training mice for H-2 odortype discrimination, and to degrees of concordance attained in the Y-maze by trained mice, these urinary H-2 odortype sources from outbred mice were no less effective than urines customarily obtained for those purposes from non-segregating inbred donors. We conclude that discrimination of H-2 odortypes is not appreciably affected or impaired by the usual concurrent segregation within the genome as a whole.

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acj6, A Gene Affecting Olfactory Behavior and Physiology.
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acj6 was originally identified as an EMS-induced olfactory-defective mutant in an adult behavior assay (the chemosensory jump assay; McKenna et al., 1989). The larval olfactory response is also affected by *acj6*. *acj6*'s adult behavioral phenotype is due at least in part to an alteration in the peripheral response to odorants: the mutation causes a physiological defect in the adult olfactory organ, the antenna, as determined by electroantennograms (EAGs) (Ayer and Carlson 1991). Our lab has recently shown that the maxillary palp is a second olfactory organ in adult *Drosophila*: *acj6* flies also demonstrate defective electropalpograms (EPGs) (Ayer and Carlson 1992). Interestingly, *acj6* severely reduces the amplitude of the response to all odorants that we have analyzed except benzaldehyde (odor of almond). *acj6* therefore distinguishes two pathways for the sensation of olfactory information. The peripheral physiological defect, coupled with the possible requirement for *acj6*$^+$ function in only a subset of olfactory responses, makes *acj6* interesting for further analysis.

We have mapped *acj6* to the X chromosome between *garnet* and *scalloped* at position 49.4. Using deficiencies (deletions) and duplications localized to this region, *acj6* appears to be located to the cytogenetic region 13C-F1. We have further mapped *acj6* to within 0.02 cM (approximately 10kB) of P(*ry*$^+$)AS438, a transposable element, which lies in chromosomal band 13C1. Efforts to characterize the gene in molecular and genetic detail are underway.

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Mouse Inbred Strain Taste Sensitivity to Acids. J.D. BOUGHTER JR. and GLAYDE WHITNEY (Program in Neuroscience, Florida State University)

Six inbred strains of mice (SWR/J, C3HeB/FeJ, C57BL/6J, BALB/cByJ, DBA/2J, 129/J) were given two-bottle preference tests with 6 inorganic and carboxylic acids to examine relative sour taste sensitivity. Ascending series were conducted with hydrochloric, citric, tartaric, formic, acetic and benzoic acids. In general, all strains showed avoidance with increasing concentration. Carboxylic acids were avoided at similar concentrations as HCl but at a higher pH. Significant strain differences were found for all six acids. Select differences at particular concentrations for certain acids were re-examined for robustness.

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C3.SW Congenic SOA-taster Mice: The Taster Allele on a Demitaster Genomic Background. J.D. BOUGHTER JR. and GLAYDE WHITNEY (Program in Neuroscience, Florida State University)*

Sucrose Octaacetate (SOA) taste sensitivity is mediated by a single genetic locus, three-allele system in mice. Inbred strains can be classified according to SOA phenotype as tasters, demitasters (intermediate sensitivity), or nontasters. C3.SW-*Soa*^{a} taster mice were bred from SWR/J (taster) and C3HeB/FeJ (demitaster) inbred strains. After 11 lineal backcross generations these congenic mice carry the tasting allele on a 99% C3 demitaster background. This model system will allow us to investigate possible pleiotropic effects of the *Soa* gene on other aspects of taste sensitivity. It will also allow comparison to existing B6.SW-*Soa*^{a} mice (nontasting background) to investigate background effects on the *Soa* gene.

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SW.B6 SOA-Nontaster Congenic Strains: Completion of a Congenic Quartet and Testing with Other Substances. DAVID B. HARDER, KIMBERLEY S. GANNON, GLAYDE WHITNEY (Department of Psychology, Florida State University)*

Six sucrose octaacetate (SOA) nontaster mouse strains congenic with the SWR/J taster inbred strain were bred via eleven backcross-intercross cycles, with selection for nontasting in each cycle. Preference ratio distributions and taster-nontaster proportions across cycles were consistent with monogenic segregation of 0.1mM SOA non-avoidance. Response functions across eleven SOA concentrations (1mM - 0.01μM) for both SW.B6-*Soa*^{b} (nontaster) and B6.SW-*Soa*^{a} (taster) congenic strains were indistinguishable from the functions of the inbred strains with the same *Soa* alleles (C57BL/6J and SWR/J respectively) despite the background genome differences. The same pattern was seen with other acetylated sugars, brucine and denatonium benzoate. It was not seen with L-phenylalanine or quinine-HCl. Acetic, citric, HCl and picric acids, sodium saccharin, thaumatin and ethanol produced different patterns as well. The effects of *Soa* allelic substitution, at least on these two genetic backgrounds, appear limited to a subset of bitter compounds.

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Strain Differences in Gustatory Responses to Amino Acids in Rainbow Trout, *Oncorhynchus mykiss*

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We have previously shown that the palatal gustatory receptors of rainbow trout (*Oncorhynchus mykiss*) respond to limited numbers of amino acids, and presented evidence indicating that they are detected through three separate receptor mechanisms: 1) proline/alanine, 2) α-aminoguanidinopropionic acid (AGPA)/betaine, and 3) leucine/phenylalanine receptors. Our recent studies have further demonstrated that proline/alanine receptors are predominant in all salmonid species (6 genera, 14 species) examined, and that some salmonids lack other receptor types and others possess either AGPA/betaine or leucine/phenylalanine receptor, or both. These results have led to a hypothesis that salmonids may have initially evolved proline/alanine and AGPA/betaine receptors and, with phylogenetic advancement, they gained greater response capabilities by (1) acquiring new receptor types, and (2) by losing the specificity of existing receptors. In the present study, we examined the gustatory responses to amino acids in different strains of rainbow trout originated from Isle of Man (U.K.), Japan, and Norway as well as several places in North America. Considerable strain differences were found, ranging from specific response to L-proline alone to wide responses to all amino acids above. No within-strain difference was found. The possibility that the observed strain differences, due possibly to a loss in genetic variation, may be a result of repeated introductions from the native range (Pacific Ocean and the coastal drainage of North America extending from Alaska southward to Mexico) and hatchery practices is discussed.

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Responses to Water by the Chorda Tympani Nerve May Be Aberrant After Regeneration. PETER CAIN and MICHAEL A. BARRY (Dept. of BioStructure and Function, University of Connecticut Health Center).

We were interested in the possibility of aberrant responses to various taste stimuli during recovery after chorda tympani (CT) crush. Dysgeusias in humans have been associated with damage to the CT from middle ear surgery or dental procedures. The CT of adult male golden Syrian hamsters (Mesocricetus auratus) was crushed distal to the ganglion, in the middle ear. After recovery periods ranging from four to sixteen weeks, the CT was exposed at its exit from the tympanic bulla to the juncture with the lingual nerve. The electrophysiological responses of the whole CT to different concentrations (in M) of sucrose (0.1, 0.3, 0.5), NaCl (0.03, 0.1, 0.3), KCl (0.1, 0.3) and a deionized water rinse were recorded. The ratios of water response to 0.1 M NaCl response from regenerated nerves were compared to those from intact animals with no damage to the CT. Typically, intact normal animals show a weak, if any, response to water. In more than 50% of the experimental animals the response of regenerated nerves to deionized water was greater than normal. The water response appeared strongest after sucrose stimulation. These responses may be the result of stimulation of a particular class of fibers or all fibers. Further investigation using single fiber techniques might elucidate the effect of these responses on taste perception.

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TASTE RESPONSES FROM THE CHORDA TYMPANI NERVE IN THE SENESCENCE-ACCELERATED MOUSE.

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Recent rodent behavioral and electrophysiological studies indicate small changes in taste as a function of age. Apart from the lack of age-related loss of taste buds, there is a possibility that the age-related loss of taste acuity might occur in the peripheral taste system as a result of decreased sensitivity of taste buds to gustatory stimuli. Such changes should be reflected in electrophysiological responses from taste nerves. It is well established that the SAMP1TA/Aud and SAMP8/Ta//Aud strains with accelerated senescence and age-related pathologies appear to be a sole animal model available for research on aging. In the present study, 2 strains of this animal model using (three different strains of mice(Slc:ICR, SAMP1TA /Aud and SAMP8/Ta//Aud)) taste sensitivity was studied by analysis of integrated responses of the chorda tympani nerve to various taste stimuli(sweet: sucrose, salty: NaCl, sour: HCl and bitter: QHCl). We compared results obtained from comparable age groups of these strains of mice. The results were as follows, 1)SAMP1TA/Aud mice possess the high sensitivity to HCl, NaCl and QHCl, and the low sensitivity to sucrose, 2) SAMP8/Ta//Aud has high sensitivity to HCl and NaCl, and low sensitivity to sucrose and QHCl, and 3)Slc:ICR shows high sensitivity to HCl, NaCl and sucrose, and low sensitivity to QHCl. These response characteristics were compared among the 3 strains examined.

Diet-Induced Plasticity in Taste Cells Modifies Feeding in an Insect Model, *Manduca sexta*

JOHN I. GLENDINNING (University of Arizona)

In contrast to visual and auditory receptor cells, chemoreceptor cells (in both vertebrates and invertebrates) display an extraordinary degree of plasticity in their responsiveness to stimuli. This plasticity is unrelated to the short-term adaptation typical of sensory cells because it occurs over several days. Despite several reports of this phenomenon, its functional significance remains unclear. In this study, I asked whether long-term sensitivity changes in the taste cells of *Manduca sexta* caterpillars caused measurable changes in feeding behavior. More specifically, I examined the effects of two days of dietary exposure to caffeine, a compound that stimulates one of the four taste cells (the deterrent cell) located within the lateral styloconic sensilla of *M. sexta*, on the physiology and behavior of the animals. Caffeine is unpalatable and *M. sexta* normally rejects food containing it. I hypothesized that prolonged exposure to caffeine would (1) decrease the responsiveness of the deterrent cell to both caffeine and another related compound, salicin, in a specific manner (i.e., without affecting the responsiveness of the other taste cells to their respective 'best' stimuli); and (2) eliminate the normal behavioral rejection response to diets treated with caffeine or salicin. My preparation enabled me to make repeated electrophysiological recordings from the same deterrent cell before and after dietary manipulations, make comparisons across individual caterpillars using homologous taste cells, and relate sensitivity changes within the deterrent cell to feeding behavior in the same animal. I found strong support for both hypotheses, indicating that the peripheral sensitivity changes are specific to the deterrent cell and that they are of sufficient magnitude to alter feeding behavior. These findings point to a complex inter-relationship between recent dietary history and food preference.

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Timecourse of Saline-Induced Recovery of the Gustatory System in Sodium-Restricted Rats.

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DAVID L. HILL (University of Virginia).

Placing pregnant rats on a Na^+-restricted diet (0.03% NaCl) on or before postconception day 8, and then maintaining the offspring on that diet, results in greatly reduced chorda tympani responses to Na^+ stimuli in the offspring during adulthood. Normal chorda tympani responses to Na^+ stimuli can be permanently restored by providing Na^+-restricted rats one-time access to at least 30 mls of isotonic saline. This effect is observed many weeks after saline ingestion, indicating that receptor cell precursors are influenced by the manipulation. In the present study, we examined the rapid timecourse of saline-induced taste system restoration. Chorda tympani responses were obtained from Na^+-restricted rats 2h, 6h, 24h, 10 days and 20 days following saline ingestion. Chorda tympani responses obtained from rats at 2h, 6h and 24h after saline intake were comparable to responses from control Na^+-restricted rats. Similarly, chorda tympani responses from rats 10 days following access to saline were not significantly different from those of age-matched controls. Remarkably, only 20 days after saline consumption were normal chorda tympani responses to Na^+ stimuli observed; responses were similar to Na^+ responses from rats bred and raised on normal Na^+ diet (1.0% NaCl). These results indicate that extant taste receptor cells are not substantially influenced by the saline ingestion. Furthermore, the delayed, abrupt appearance of normal chorda tympani responses to Na^+ stimuli 20 days after saline intake suggests that taste receptor stem cells are influenced by this manipulation only during a highly discrete phase of the cell cycle. Alternatively, normally cycling stem cells may somehow be 'phase-locked' and subsequently cycle in unison to produce normally functioning taste receptor cells. Finally, candidate humoral factors which could prompt taste system recovery in this paradigm must be limited to factors which exert their actions exclusively on the stem cell genome.

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Development of the Enhanced Neural Response to NaCl in Fischer 344 Rats.

SUZANNE I. SOLLARS, GLENN E. SCHAFE & ILENE L. BERNSTEIN (University of Washington).

Adult Fischer 344 (F344) rats fail to prefer any concentration of NaCl to water and avoid those concentrations (0.6 - 1%) maximally preferred by other strains such as the Wistar rat. The chorda tympani (CT) neural response to NaCl is significantly higher in F344 versus Wistar rats, a difference which is eliminated by preapplication of amiloride to the tongue. The following study examined the development of the strain differences in the CT response to NaCl. Wistar and F344 rats were tested in three age groups: 15-17, 21-23, and 34-36 days of age. Whole-nerve-integrated CT responses were recorded before and after lingual application of amiloride. NaCl response magnitudes were expressed as ratios relative to ammonium chloride (NH_4Cl) since responses to NH_4Cl are unaffected by amiloride. At 15-17 days of age, there were no significant strain differences in the magnitudes of NaCl responses at any concentration tested. Strain differences emerged at 21-23 days of age with the relative response ratios in the F344 rats significantly higher than those of the Wistar rats. These differences became more pronounced in the 34-36-day-old group. Within each age group, amiloride significantly reduced responses to NaCl and eliminated any differences between the strains. The changes in neural responsiveness correspond with the emergence of the behavioral expression of F344 salt aversion. These results are consistent with the interpretation that amiloride-sensitive sodium channels have a role in the development of differences between F344 and Wistar rats' CT responses to NaCl.

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Withdrawn

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Taste Papilla Morphogenesis in Rat Tongue Organ Culture. J.P. MBIENE (School of Dentistry), D. MACCALLUM (Medical School) and C.M. MISTRETTA (Dentistry, Univ. of Michigan, Ann Arbor, MI 48109).

During development of the taste system, fungiform and circumvallate papillae make a morphological appearance in the rat tongue between embryonic days 14 and 15 (E0: dam is sperm positive). From time of initial formation, fungiform papillae are organized in rows on either side of the midline of the anterior tongue and the single circumvallate papilla is located in the middle of the posterior oral tongue. The spatial patterns and morphological stages of development of these papillae are well documented. However, beyond these basic facts little is known about factors that regulate taste papilla morphogenesis. Therefore, we have used an organ culture system to learn whether the embryonic rat tongue can be maintained in vitro and, if so, whether tongue and papilla development can proceed. E13 and E14 embryos were obtained from anesthetized, pregnant Sprague-Dawley rats. Embryonic tongues were dissected from the oral cavity, placed on filter paper, and maintained at the interface between the gas and liquid phases of a standard organ culture. Organ cultures were incubated at 37°C, 100% humidity in 5% CO_2 in air. The medium was DMEM/F12 (1:1) containing a culture supplement, B-27 (Gibco), 1% fetal bovine serum and 20 µg/ml gentamicin SO_4. Cultures were fixed after 1, 2, 3 and 6 days and processed for light and scanning electron microscopic analysis. General tongue morphology progresses through appropriate developmental stages and the tongue increases in size in culture. Fungiform papillae develop in roughly patterned rows on both sides of the midline of the anterior tongue after 2 and 1 day(s) of culture, respectively, for E13 and E14 explants. Fungiform papillae are still maintained after 6 days of culture. Light microscopic analysis confirms the presence of a mesenchymal core in the fungiform papillae characteristic of in vivo development. Circumvallate papilla development is inconsistent. These results indicate that: rat embryonic tongue can be maintained under culture conditions; tongue development progresses in culture; and, the embryonic tongue in culture exhibits spatial patterns and morphogenesis of fungiform papillae similar to those observed in vivo. The culture system should be suitable for further investigation of regulatory factors in rat gustatory papilla development.

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Multiple Progenitors from Local Epithelium Form Taste Buds in Mice.

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Two fundamental questions about taste bud development were addressed in this study: 1) Do taste cells arise from local epithelium or do taste cell progenitors migrate into the epithelium during development? 2) Are the 50-150 cells within each taste bud derived from a single progenitor, or from multiple progenitors? To investigate these questions we examined the taste buds of fungiform and circumvallate papillae and surrounding epithelium of X-inactivation mosaic mice. One of the two X chromosomes in these female mice carries multiple copies of the promoter of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA) linked to the *E.coli lacZ* gene. Early in development, either the transgenic X chromosome or the non-transgenic X chromosome is randomly inactivated in each cell. Once X inactivation occurs, it is a stable and heritable feature of that cell and can be used in cell lineage analyses; cells retaining the active *E.coli lacZ* gene are identifiable using a histochemical reaction which stains them blue. Previous studies and our results indicate that X inactivation in lingual tissue occurs before taste bud progenitors are committed. Serial sections were stained with XGal to identify cells with the active transgene, then counterstained with neutral red or nuclear fast red. The lingual epithelium of these mice consists of patches of transgenic (blue) and non-transgenic (red) cells. Taste buds contained entirely within transgenic patches only contain transgenic cells while taste buds in non-transgenic patches only contain non-transgenic taste cells. Thus the taste bud phenotype always matches that of the surrounding epithelium. Analysis of taste buds on borders between transgenic and non-transgenic patches reveals that these taste buds may contain both transgenic and non-transgenic cells. Thus taste buds are not clonal. In conclusion, taste bud cells arise from local epithelium since the taste bud population always reflects that of the surrounding epithelium. In addition, more than one progenitor contributes to each taste bud population since both transgenic and non-transgenic cells can be found in single taste buds.

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The Distribution of Tenascin and Syndecan during Fungiform Papilla Morphogenesis. J. MORRIS-WIMAN and L. BRINKLEY (University of Florida Dental College)

The developmental sequence of fungiform papilla patterning and formation resembles that of other placode-derived structures such as hair and teeth, feathers and scales, that arise as a result of epithelial-mesenchymal interactions. In tooth and whisker follicle formation in the rodent, two extracellular matrix molecules, tenascin and syndecan, are believed to play a major role in mediating these interactions (see Bernfield and Sanderson, Phil Trans R Soc, 1991). To determine if these molecules have a role in fungiform papilla patterning and formation, their distributions were examined using standard immunofluorescent-staining techniques during papilla morphogenesis in the fetal mouse tongue (gestational days 11 to 17). Both tenascin and syndecan were localized within the lateral mesenchyme and the dorsal epithelium of the merging processes. By the completion of tongue formation, tenascin had disappeared from the dorsal epithelium and syndecan was lost from the mesenchyme. At this time, tenascin was sparsely distributed throughout the dorsal mesenchyme. With placode formation, an increased intensity of staining for tenascin was observed within the dorsal tongue mesenchyme. Interestingly, tenascin was lost from the dorsal mesenchyme subjacent to the placode as the placode acquired a mesenchymal core, although it remained abundant in the adjacent mesenchyme. Prior to placode formation, syndecan was observed on lateral and apical cell surfaces of the dorsal tongue epithelium. The formation of the placode was associated with the loss of syndecan from the lateral surfaces of epithelial cells forming the placode. In the forming papilla, syndecan was more abundant on the basolateral surfaces of apical epithelial cells. Tenascin had an increased expression subjacent to the apical epithelium, although tenascin was observed throughout the papillary epithelial basement membrane. The observation that distinct temporospatial distributions exist for tenascin and syndecan that correlate with stages in the patterning and formation of fungiform papilla suggest that these molecules may play a role in papilla morphogenesis.

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Structural aspects of vertebrate taste organ phylogeny.

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During the last three decades a lot of information concerned to the ultrastructure and histochemistry of vertebrate taste organs (TOs) was brought together. Most of it is concerned to fish and mammalian taste buds (TBs) and it is generally accepted that a fish TB structurally is not the same as a frogs taste disk (TD) or a mammalian TB. In view to other vertebrate groups our respective knowledge is rather poor. These gaps are concerned to the TOs of numerous and ecologically highly specialized animals, as groups of reptiles and birds. Insofar it is very difficult to work out something like the vertebrates TO-phylogenetic system. – By comparison of structural details and histochemical findings from TOs from vertebrates of different systematic position, as the TO´s sensory epithelium including the chemoreceptive cells and their apical endings within the TO´s receptor area, the basal cells and especially the TO´s synaptic connections, to some extent allow an interpretation of TO-evolution. But, on the other hand, the ecological situation of the respective animals must be regarded too: Structural details may be altered by adaption and therefore are secondarily acquired. So it looks like that vertebrate TOs were not established in a monophyletic way. It is more likely that vertebrate taste organ evolution is to be represented as an arborized, polyphyletic tree.

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Ultrastructure of the taste disk of the African clawed frog, *Xenopus laevis*.

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The taste disk (TD) of the African clawed frog has been investigated by scanning and transmission electron microscopy and compared with that of other Salientia. Unlike in tongue-bearing species, the TD´s of *Xenopus* are especially found in the mucosa of the mouth floor and the palate. They are not situated on the top of fungiform papillae. TD´s are surrounded by specific electron-lucent cells containing some secretory vesicles. The TD´s epithelium consists of mucus cells, wing cells, sensory cells and basal cells. The mucus cells are arranged in two layers: One is lining most of the surface area (as usual in other frogs), and the other layer is situated directly beneath the first and may reach the basal membrane. Mucus cells possess an unregularly shaped nucleus and two populations of secretory vesicles, granulated and electron-lucent ones. The slender sensory cells reach from the basal membrane up to the TD´s surface, the receptor area. Sensory cells apically bear either one rod-like process or a tuft of small microvillar processes. Sheet-like processes of wing cells embrace both superficial mucus cells and sensory cells. On the TD´s base some electron-lucent processes of (degenerating?) sensory cells with numerous dense-cored vesicles are located immediately above the nerve fiber plexus. They show, as well as the more electron-dense cell processes of other sensory cells, synapses to the plexus´ nerve fibers. Merkel cell-like basal cells with short spine like processes contain clusters of dense-cored vesicles and intermediate filaments. -- Taken together, the TD of *Xenopus* differs from that of the Ranidae in that they have a different equipment with mucus cells and no ciliated cells surrounding the TD. The structure of wing cells, sensory cells and basal cells is similar to that as observed in other frog and toad TD´s. Insofar, *Xenopus* as a popular laboratory animal may also serve for chemoreception tests and replace the highly protected *Rana* species.

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Hypoglossal Neural Activity During Taste-Elicited Rejection Responses in the Awake Rat

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Behavioral and electromyographic (EMG) studies indicate that the lingual musculature participate in the ingestion and rejection of taste stimuli (Grill and Norgren '78; Travers and Norgren '86). The role of single hypoglossal motoneurons (mXII) in producing the appropriate response, however, is unknown. This study examined the characteristics of single mXII cells during ingestion (licking and swallowing) and rejection (gaping) of gustatory stimuli in a chronic preparation to determine if cells are more specialized for one response over the other. Licking bouts were initiated by intra-oral infusions of water, sucrose, and sodium chloride, while gapes were induced by quinine monohydrochloride (QHCL). Comparison between neural activity and behavior was accomplished by recording single mXII cells simultaneously with EMG recordings from 3 oro-pharyngeal muscles. The temporal characteristics of mXII cells were determined by cross-correlating unit activity with rhythmic jaw opening (anterior digastric EMG). The temporal re-ordering of lingual muscle activity during gapes was observed at the single motoneuron level as phase shifts in the cross-correlations. Phase shifts elicited by QHCL were confirmed in a sample of mXII cells in which videographic records of lingual movements were directly compared to patterns of neural activity. Neural activity was quantified by counting the number of spikes per lick, swallow, and gape for 32 mXII cells. Cells were characterized as either excitatory or inhibitory during swallows or gapes if the number of spikes deviated by 25% or more from the number of spikes per lick cycle. Both excitatory and inhibitory responses were evident during gapes (excit. = 66%; inhib. = 22%) and swallows (excit. = 22%; inhib. = 6%) suggesting specialization in the motoneuronal pool. Nevertheless, a scatterplot of the number of spikes during gapes versus swallows showed no consistent relationship. Thus, although subsets of mXII cells specialized for different components of ingestion and rejection were evident, excitation or inhibition of a single cell during ingestion did not predict a cell's response during rejection.

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Northern and Western Analysis of Interleukin 1-ß Expression in Mouse Olfactory Epithelial Tissue.

Andrea Delkescamp and Joel Maruniak

Interleukin 1-ß is expressed in traumatized regions of mouse olfactory epithelial tissue. Naris closure and or bulbectomy resulted in IL-1ß being expressed on the open side or side subjected to bulbectomy when analyzed immunohistochemically. Using monoclonal and polyclonal antiserum, we have found that in normal tissue, IL-1ß is secreted by deep Bowman's glands. Following naris closure, IL-1ß immunoreactivity appeared in the secretory regions of the olfactory epithelial supporting cells as well as the Bowman's glands.

Northern and Western analysis will conclusively confirm the presence of IL-1ß in traumatized regions of olfactory epithelial tissue.

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Chemical Modification of Brazzein, a Sweet Protein from *Pentadiplandra brazzeana*

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Brazzein is a sweet protein isolated from *Pentadiplandra brazzeana*. It has been purified and characterized in this laboratory. This protein is rich in lysine, tyrosine, glutamate, aspartate. No tryptophan, methionine and threonine have been found in this protein. Chemical modification has been carried out in this laboratory to detect the roles of the specific amino acids in the process of sweet taste. The chemical modification of Brazzein includes reductive methylation, acetylation, succinylation and pyridoxal modification of lysine residues; iodination and O-acylation of tyrosines; S-pyridylethylation and carboxymethylation of cysteines; p-hydroxyphenolglyoxal and 1,2-cyclohexanedione modification of arginines; amidation of carboxyl groups; and diethylpyrocarbonate modification of histine residues in the protein. The results show that arginines, but not lysines, may involved in the interaction of this sweet protein with sweet receptor(s). Like Thaumatin and Monellin, the tertiary structure of native Brazzein is essential for the elicitation of sweetness.

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