AChemS-XI

The Eleventh Annual Meeting of the Association for Chemoreception Sciences

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MORNING

Thur

Talk | Poster

7:30 Continental Breakfast

8:00 — Development #1 - 7 — 7 papers

8:00 -12:00 — Receptor Cell Function I — #16-37 — 22 papers

9:45 Refreshment Break

10:15 — Psycho-physics I- Clinical — #8-15 — 8 papers

10:15 — Chemical Ecology — #38-42 — 5 papers

12:30 Executive Committee Meeting

Fri

Talk | Poster

7:30 Continental Breakfast

8:00 -12:00 — Psycho-physics III — #86-99 — 14 papers

10:00 Refreshment Break

10:30 Jack Pearl

11:00 — Symposium 2: Perireceptor Events — #80-85

11:00 — Central Gustatory Pathways I — #100-113 — 14 papers

Sat

Talk | Poster

7:30 Continental Breakfast

8:00 — Receptor Cell Function IV — #147-154 — 8 papers

8:00 - 12:00 — Central Olfactory Pathways II — #163-187 — 25 papers

10:00 Refreshment Break

10:30 — Psycho-physics V: Classical — #155-162 — 8 papers

12:30 AChemS Business Meeting

Sun

Talk | Poster

7:30 Coffee and Juice

8:00 — Receptor Cell Structure II — #196-204 — 9 papers

8:00 — Psycho-physics VII — #205-220 — 16 papers

10:15 - 11:30 **BRUNCH**

EVENING

Wed

5:00 - 7:30 Registration

5:30 Meeting for Graduate Students Receiving Travel/Housing Awards

6:00-7:30 Opening Buffet

7:30 Welcome, Opening Remarks, Awards

8:00 Givaudan Lecture

9:00 Social Reception Cash Bar

Thur

Talk | Poster

4:00 Symposium 1: Chemical Gradients and Chemical Sensing #43-46

6:00 Central Olfactory Pathways I #47-50 4 papers

9:00 Break

9:30 Psycho-physics II: Food Related #51-54 4 papers

7:00 - 11:00 Receptor Cell Function II #55-65 11 papers

7:00 - 11:00 Animal Behavior #66-79 14 papers

Fri

Talk | Poster

7:00 Central Gustatory Pathways II #114-119 6 papers

8:30 Break

9:00 Receptor Cell Function III #120-125 6 papers

7:00 - 11:00 Receptor Cell Structure I #126-139 14 papers

7:00 - 11:00 Psycho-physics IV- Sensory Evaluation #140-146 7 papers

Sat

Talk

7:00 Psycho-physics VI- Olfaction #188-191 4 papers

8:00 Break

8:30 Symposium 3: Leukocyte Chemotaxis #192-195

1

Differentiation *in vitro* of Cultured Olfactory Neuroblasts R. D. SWERDLOW, A. DEGRASSI, & H. G. COON (Lab of Genetics, NCI, NIH, Bethesda, MD)

The basal cells of mammalian olfactory epithelium (OE) are capable of dividing and differentiating into mature sensory neurons throughout the life of the individual. Although this fact is widely recognized, little is known of what events are involved at the cellular level in initiating this process, or of the molecular mechanisms responsible for the cell's transition from a replicative stem cell to a mature neuron. Using lines of cultured cells, derived from rat OE, previously shown to respond to some odorants by producing cAMP (Coon et. al. 1989 PNAS, in press), we describe that some of these cells can also be induced into an *in vitro* process resembling neuronal differentiation. When grown in protein rich medium, these cells divide continuously, and have a flat epitheloid shape. They lack, or express at low levels, several neuron specific marker proteins. Upon replacing the medium with protein free medium, the cells stop dividing and begin to acquire the distinct morphology of neurons: rounded cell bodies with elongated bipolar projections. Neuron specific proteins appear at increasing levels, in some cases transiently during the first week. After two weeks differentiation appears to be completed. These cells can remain in this differentiated state for as long as two months. Using the appearance of neuron specific markers as a relative index , we evaluated the effects of nerve growth factor, basic fibroblast growth factor, OE extract, and retinoic acid added to the differentiation medium.

2

Organization of Astrocytes in the Developing Rat Olfactory Bulb M.S. BAILEY, M.T. SHIPLEY (Dept. of Anatomy and Cell Biology) and R.A. AKESON (Division of Basic Research, Children's Hospital Research Foundation, Univ. of Cincinnati Medical Center, Cincinnati, OH)

Monoclonal antibodies 2C8, 2F5, 1F10 and polyclonal anti-GFAP antibodies (DAKO and L. Eng) label astrocytes in the rat CNS. All of these antibodies demonstrate a differential laminar distribution of astrocytes in the adult rat olfactory bulb. In addition, 2C8 lightly labels mitral and tufted (M/T). We report the first studies on the patterns of astrocytes in the developing olfactory bulb and the developmental expression of the 2C8 antigen.

Post-natal day 2,5,9, and 16 animals were examined. In P2 bulbs, the region deep to the mitral cell layer has labeled astrocytes, though the greatest density of anti-GFAP labeled astrocytes is in the glomerular layer (GL). Between these two regions are prominent radially arrayed fibers. 2C8, in P2 bulbs, lightly stains some M/T cells; in addition, 2C8 stains astrocytes although much less densely than does anti-GFAP. At P5 and P9 the intensity of M/T cells labeled with 2C8 increases relative to the labeling of astrocytes and radial fibers. At P9 the M/T cells are strikingly positive for 2C8. 2F5 and 1F10 preferentially label astrocytes in the GL at all early ages. By P16 all antibodies, including 2C8 stain astrocytes in a pattern very similar to the adult.

The relative density of GFAP expression in the glomeruli appears to correlate in location and time with synaptogenesis. Mabs 2F5 and 1F10 may identify GFAP epitopes that are expressed later than those recognized by polyclonal anti-GFAP. Mab 2C8 is intriguing. It appears to recognize an epitope which is present in mature astrocytes and whose peak expression in M/T cells is episodic in development. The peak expression of 2C8 at P9 does not correlate with neurogenesis of M/T cells; and the absence of large labeled cells deep to the mitral cell layer suggest that 2C8 expression is not related to cell migration. We hypothesize that the 2C8 antigen has a role in M/T cell differentiation.

(Supported by NIH 5PO1NS23348 and Research Challenge, UCMC)

3

Tyrosine Hydroxylase Expression In Olfactory Bulb In Vitro Is Dependent on Receptor Afferent Innervation. HARRIET BAKER (Cornell Univ. Med. Coll.), ALBERT I. FARBMAN (Northwestern Univ.).

Recent studies strongly suggest a relationship between receptor afferent innervation and both development and maintenance of phenotype in olfactory bulb neurons. For example, synthesis of the first enzyme in the catecholamine biosynthetic pathway, tyrosine hydroxylase (TH), appears to depend on the integrity of the afferent pathway. The mammalian olfactory system presents a number of obstacles to the study of mechanisms underlying interactions between olfactory epithelium and bulb, including the fact that development is embryonic which precludes direct surgical or pharmacological manipulation. Explant cultures were recently utilized to establish the dependence of olfactory marker protein (OMP) expression on the presence of olfactory bulb. The studies reported here investigated the reciprocal relationship, namely, dependence of TH expression in olfactory bulb on the receptor epithelium. Explants prepared from E15 olfactory bulb alone or en bloc with receptor epithelium were analyzed after 7 days in vitro. The en bloc cultures contained numerous TH-stained cells both in large clusters and scattered throughout the bulbar tissue. The cells were in close proximity to OMP-containing fibers, but not necessarily in a periglomerular position. Bulb alone cultures exhibited only a few lightly TH-stained cells usually in one location in the explant. Explants, prepared by separating the epithelium from bulb and then culturing them together, contained an intermediate number of TH cells suggesting either that reinnervation was incomplete or that the perturbation delayed maturation of the system. These studies demonstrate that in vitro as in vivo expression of the dopamine phenotype is dependent on olfactory receptor afferent innervation. Supported by Grant #'s NS23103 & NS06181.

4

Developmental Change in Olfactory Habituation is Mediated by Anterior Commissure Maturation.
W.G. HALL (Duke University)
CYNTHIA HEDRICK (Duke University)

Rat pups orient to the presentation of a novel odor by raising their heads and sniffing. With repeated exposures, this orienting response habituates (Siegel et al, Psychobiol, 15:122-7,1987). Such habituation of orienting was used to study the ontogeny of cross-projections in the olfactory system and their role in memory storage and access. Six- and 12-day-old pups received training or testing on one side of their olfactory system by blocking the contralateral naris with a soft rubber plug. Training consisted of 15, 10-sec presentations of odor (orange or peppermint) at 1 min intervals. Later, pups were tested with 15 additional presentations of the same odor used in training. We found that, during training, pups of both ages first oriented to the odor and then habituated, showing little or no orientation by the end of the training trials. Six-day-old pups subsequently tested with the same naris open remembered the training and showed little response, but 6-day-old pups tested with the plug switched responded as much during testing as training. In contrast, 12-day-old pups showed good habituation memory, regardless of whether they were tested on the same or opposite side from training. This shift from unilateral to bilateral habituation memory is consistent with the known maturation of the anterior commissure (Schovb & Price, J Comp Neurol, 233:203-22,1984). We confirmed the role of the anterior commissure in mediating the bilateral habituation of 12-day-old pups by showing that when this pathway was transected 2 days before training, habituation memory did not transfer to the untrained side. With transected commissures, responses of 12-day-old pups resembled those of 6-day-old pups. Finally, by transecting the commissure after training but before testing in 12-day-old pups, we determined that the mature commissure permits bilateral storage of the habituation memory at the time of training.

(Supported by NICHD grant HD17458)

5

Early Development of Fungiform Papillae in Rat Embryos. JOSEPH PASCAL MBIENE and ALBERT I. FARBMAN, (Northwestern University, Evanston, IL 60208).

Fungiform papillae on rat tongues were studied at the earliest stages of their formation in embryos at 16 and 17 days of gestation. Observations with the scanning electron microscope revealed dome-shaped protuberances aligned on both sides of the tongue midline. At E16, the surface morphology of the papilla cells was not distinguishable from that of the surrounding cells, but by E17, the papilla cell surface was smooth, whereas the surrounding cells had many surface microvilli. When we examined thin sections of papillae with the transmission electron microscope, we saw closely packed epithelial cells containing more organelles than surrounding epithelium. To determine whether the formation of these papillae came about as a consequence of localized cell proliferation, or simply by aggregation at specific loci, we did autoradiographic studies after an injection of tritiated thymidine on E16 and E17. There was no incorporation of the labeled compound by the cells constituting the closely packed epithelium in the fungiform papillae, but there was abundant uptake by cells in the surrounding epithelium. The EM and autoradiographic data lend support to the notion that the epithelial cells of the papilla are different in nature or origin from the other epithelial cells lining the tongue. With respect to the innervation of the papillae, our findings indicate that nerve endings are not detected until E17 near, but not within, the papilla. These data are consistent with the idea that the early stages of fungiform papilla formation occur in the absence of nerve endings. Perhaps the closely packed epithelial cells act as specific target loci which attract nerve terminals. This does not rule out the possibility that nerve endings have a role in later stages of taste bud differentiation. However, the first events of fungiform papilla formation apparently occur independent of nerve terminals.

6

Laryngeal Taste Buds in the Hamster: Morphological Development and Chemical Sensitivity. DAVID V. SMITH, TERI L. BELECKY (University of Cincinnati College of Medicine), and TAKAMITSU HANAMORI (Miyazaki Medical College)

Taste buds in the larynx, distributed on the laryngeal surface of the epiglottis and on the aryepiglottal folds, comprise about 10% of the total number of taste buds in adult hamsters (Miller & Smith, 1984). These chemoreceptors, innervated by fibers of the superior laryngeal nerve (SLN), may play a role in neonatal reflex apnea in a number of nonrodent species (e.g., Boggs & Bartlett, 1982; Storey & Johnson, 1975). Laryngeal taste buds were quantified in postnatal hamsters of 1 to 120 days of age from paraffin-embedded tissue cut at 8um and stained with Heidenhain's iron-hematoxilyn and eosin. Counts of taste buds were made from successive serial sections and both immature (i.e., buds without pores) and mature buds were counted. Similar to taste buds innervated by the hamsters' IXth nerve (Miller & Smith, in press), development of laryngeal taste buds was entirely postnatal, with no buds evident at birth. The postnatal growth of these buds was a logarithmic function of age ($Y = 63.6\log X - 2.25$). At the earliest postnatal ages (under 10 days), only about half of the laryngeal taste buds had a distinct pore. By 90 days of age, all of the taste buds were mature, although their numbers continued to increase. Responses were recorded from 65 SLN fibers innervating these laryngeal taste buds in adult hamsters. Of the 65 fibers, 56 responded to water and to hypotonic concentrations of NaCl and 55 responded to hypertonic NaCl solutions. Most fibers (44/65) responded to water, hypotonic, and hypertonic NaCl. A few fibers (10/65) responded only to water and hypotonic NaCl and a few (9/65) only to hypertonic NaCl. The mean time course of these fibers to distilled water and hypotonic NaCl solutions was similar and distinct from that to hypertonic NaCl solutions, suggesting different receptor mechanisms for these two classes of stimuli. The distribution of these sensitivities across SLN fibers suggests that the receptor mechanisms for hypotonic and hypertonic stimuli are distinct from one another but that their input to these fibers largely overlaps. This results in a set of sensitivities uniquely adapted to signal deviations from the *internal milieu* of the larynx. Since it has been shown that SLN chemosensitivity derives from stimulation of laryngeal taste buds (Stedman et al., 1980), chemosensory influence over airway-protective reflexes is probably not fully functional until after weaning in hamsters.

Supported by NINCDS Grant NS23524-03 to D.V.S.

7

Neural Control of the Expression of Filiform Papillae in Adult Rat Tongue

BRUCE OAKLEY, ANNE LAWTON and LAN-HSIN WU, (Biology Dept., Neuroscience Lab. Bldg., Univ. of Michigan, Ann Arbor, MI 48109)

In normal tongues each fungiform papilla has a smooth apical surface free of filiform papillae. We examined serial sections of rat tongues from 8d to 6mo after unilateral transection of the chordalingual nerve (control hypoinnervation, n=7 rats) and 6-8mo after either transection of the chorda tympani nerve (n=5) or experimental innervation by the mylohyoid nerve, lingual+mylohyoid or lingual+auriculotemporal nerves (n=14). Chordalingual transection totally eliminated nearly half of the fungiform taste buds. By 6mo 60% of these empty fungiform papillae had an extraneous filiform-like papilla growing from the apical surface. In contrast, a filiform outgrowth was never observed on fungiform papillae containing an atrophic or remnant taste bud sustained by reinnervation. Measurements from SEMs revealed that the cross-sectional area of the trigeminal nerve branches was linearly related to a reduced incidence of extraneous filiform papillae. With maximal trigeminal innervation only 30% of the empty fungiform papillae had an extraneous filiform papilla. The chorda tympani nerve was about 50 times more effective than trigeminal branches in preventing filiform expression. We suggest that normal innervation by gustatory axons simultaneously promotes the expression of taste buds and prevents the expression of filiform papillae. Trigeminal axons have a much weaker, but clearly demonstrable, capacity to suppress filiform outgrowth.

Supported in part by NIH Grant NS-07072.

8

Psychophysical Mapping of a Human Case of Left Unilateral Ageusia. **CARL PFAFFMANN** (The Rockefeller University, New York, NY 10021) **LINDA BARTOSHUK** (Pierce Foundation & Yale University, New Haven, CT 06519)

A case (C.P.) of Herpes zoster oticus (a viral infection of cranial nerves) presented with unilateral (left) auditory, facial, and glossopharyngeal nerve damage. We present here a progress report on a psychophysical measurement of loss and recovery of taste sensitivity mediated by the facial and glossopharyngeal nerves. We measured taste function with a spatial Q-tip test developed for the Connecticut Chemosensory Clinical Research Center (CCCRC). This test consists of "painting" strong solutions of NaCl (1.0 M), sucrose (1.0 M), citric acid (0.032 M), and quinine hydrochloride (0.001 M) on localized regions of the left and right sides of the tongue, rear edges of the tongue, and the palate. In addition localized stimulation of the tongue surface with an electrogustometer was employed. Intensity of taste sensation is judged on a 9 point scale with 1 = very weak, 5 = medium, and 9 = very strong. The viral infection began in late April 1988. Psychophysical testing began in July. At that time there was no taste function on the left side of the palate and tongue. Incidentally, the sharp boundary and normal function on the right side of the palate suggests that the palate is unilaterally innervated in the human. As of now (December, 1988) recovery has progressed to the glossopharyngeal and greater superficial petrosal nerves but not to the chorda tympani. Additional data will be presented in April at AChemS.

9

A technique for recording the human electrogustogram GERARD HECK and JOHN DESIMONE (Medical College of Virginia, Virginia Commonwealth University, Dept. of Physiology, Richmond, VA 23298, U.S.A.)

The electrogustogram (EGG) is a record of the electrical activity due to movement of ions across the gustatory epithelium. We previously recorded the transepithelial voltage changes in response to tastants from dog and rat epithelia in vitro, and have now done so noninvasively in humans. The voltage between the interior of a vacuum-attached chamber and a reference electrode on the cheek is recorded. This voltage changes in response to tastants. A vacuum ring molded into the face of the stimulating chamber isolates a circular patch of the tongue epithelium hydraulically and electrically. Taste is stimulated in this patch by fluid flowing in from a central port and radially outward over the surface of the tongue to an annular collecting ring. A second electrode in the chamber passes current to measure transepithelial resistance (the voltage change per unit of current across the epithelium). The human EGG shows the same features found in animal experiments: voltage increases and transepithelial resistance decreases as NaCl concentration increases. Under stimulation with 1.0 M NaCl the potential reaches as high as 50 mV. Different tastants such as sugars, salts and acids produce different EGG waveforms. Our previous work established a correlation between transepithelial electrical parameters and peripheral taste function. Since psychophysical taste responses can be determined simultaneously with EGG records, this technology may prove useful clinically in distinguishing between peripheral and central lesions.

This work was supported by grants from Campbell Institute for Research and Technology and Virginia's Center for Innovative Technology

10

A Discrepancy between Odor Thresholds and Identification in Dysosmia. B.J. COWART[1,2], B. GARRISON[1], I.M. YOUNG[2] & L.D. LOWRY[2] ([1]Monell Chemical Senses Center and [2]Jefferson Medical College)*

Both threshold and identification measures are frequently used in the diagnosis of olfactory dysfunction. Results from these two types of tests typically agree, but when they are discrepant, patients are likely to appear more "normal" on threshold than on identification measures (Cain et al., Laryngoscope 98:83-88, 1988). This finding suggests identification is the more sensitive clinical measure. It could also be less specific, however. That is, the two tests might be equally sensitive to simple olfactory loss, with the identification task also being sensitive to disruptions in odor quality encoding that produce dysosmia rather than hyposmia or anosmia. We examined that hypothesis in a sample of 116 patients who presented with an olfactory complaint and scored abnormally low on an odor identification task; 81 complained only of loss and 35 complained of smell distortions. Correlations between each of two odor thresholds and the identification score were highly significant among patients reporting simple loss ($p<.001$) but not among those reporting distorted sensations ($p>.2$). In addition, patients with essentially normal threshold values were significantly more likely to report dysosmia than were those with elevated thresholds ($X^2=30.4$, $p<.001$; 78% were assigned to the correct complaint category). Thus, the discrepancy between threshold and identification measures may provide a clinical marker for dysosmia and allow for the more accurate characterization of the nature of olfactory dysfunctions.

*Supported by NIH P50 NS19616.

11

Olfactory Deficits in Allergic Rhinitis. ALLEN M. SEIDEN, ALLEN LITWIN, and DAVID V. SMITH (University of Cincinnati College of Medicine)

Nasal and sinus diseases are frequent etiologies in patients with primary olfactory complaints (Goodspeed et al., 1987; Smith et al., 1987). In particular, allergic rhinitis, with or without nasal polyposis, is a well known cause of anosmia or hyposmia (Fein et al., 1966). Olfactory loss in allergic rhinitis is assumed to result from disruption of airflow to the olfactory epithelium and even minor inflammation in the ostiomeatal complex is enough to produce severe olfactory impairment (Seiden & Smith, 1988). Little is known about the frequency of olfactory deficits in nasal allergy sufferers. Patients presenting with nasal symptoms to a private allergy clinic were evaluated with skin testing for IgE-mediated inhalant allergy and for smell function using the University of Pennsylvania Smell Identification Test (UPSIT). Of the 37 patients who have been included in the study to date, 34 tested positive (i.e., were atopic), with diagnoses of allergic rhinitis or perennial allergic rhinitis, sometimes associated with bronchial asthma and/or polyps. The other 3 patients suffered from vasomotor rhinitis. The ages of the 15 men and 19 women who were atopic ranged from 14 to 58 years ($\bar{X}$ = 25.7). Although none of the patients presented because of an olfactory complaint, the brief questionnaire on the UPSIT indicated that 20 of the 34 allergic patients (58.8%) had experienced some "taste or smell problem". Of these 34 patients, 12 (35.3%) had a measurable smell loss, with a mean UPSIT score (21.7) significantly lower than that for those 22 patients without smell impairment (38.6, $t = 6.3$, $df = 32$, $p < .01$, one-tailed test). Eight of the patients with smell loss were hyposmic ($\bar{X}$ UPSIT score = 27.5) and four were anosmic ($\bar{X}$ UPSIT score = 10.0). Nasal polyps were evident in 6 patients and their mean UPSIT score (15.8) was significantly less ($t = 2.83$, $df = 10$, $p < .01$, one-tailed test) than for those without polyps (27.5). Although nasal allergy has been recognized as a common factor in smell dysfunction, the large percentage of allergy patients with olfactory loss (35.3%) demonstrated here is surprising. If this percentage holds with a larger number of patients, the estimates of over 20 million nasal allergy sufferers in the U.S. would suggest that about 7 million may also show olfactory deficits. Evaluation of therapy for nasal allergy (e.g., immunotherapy, pharmacotherapy, intranasal surgery) has traditionally relied on subjective measures of symptom resolution. For patients with smell loss, olfactory tests could provide an important adjunctive measure.

12

Nasal/Sinus Disease and Olfactory Loss at the Connecticut Chemosensory Clinical Research Center (CCCRC). APRIL E. SCOTT (Dept. Medicine, & CCCRC), WILLIAM S. CAIN (John B. Pierce Found. Lab., New Haven, CT and CCCRC), & Gerald Leonard (Dept. Surgery, & CCCRC).

Pathology in the nose and/or sinuses is the most commonly diagnosed etiology for olfactory loss at the CCCRC (27%, n=582). Both the CCCRC database and clinical studies are being utilized to further the understanding of diagnostic and treatment dilemmas. Diagnostically, differentiation between viral damage to the olfactory system (URI n=94) and potentially reversible disease in the nose and/or sinuses (NSD n=156) can be difficult. As sinusitis usually is preceded by a viral upper respiratory infection, the distinction can not be made solely by the history of onset of olfactory loss. Database review also reveals a significant prevalence of chronic nasal symptoms (42%), inhalant allergies (35%) and past history of NSD (25%) in the URI classified group. The URI and NSD groups differ in distributions of age and sex, severity of olfactory loss and also for the presence of rapid, spontaneous reversals of olfactory function (fluctuations). A negative ENT exam does not rule out NSD. Positive CT scans were reported in 24% of patients with normal exams. Information regarding treatment is being obtained through the database and clinical studies. Of NSD patients treated with topical corticosteroids prior to CCCRC evaluation (n=29), 62% did not respond. Of 8 patients previously treated with both topical and systemic corticosteroids, 3 responded to both, 4 neither and 1 only responded to systemic. New data from CCCRC surgically treated patients reveals a higher incidence of previous ethmoidectomies or scarring in the non-responder group. Long-term follow-up shows an 80% recurrence rate for nasal polyps and/or sinusitis for patients treated by surgery alone. From the data gathered thus far, the CCCRC recommends obtaining a CT scan if the diagnosis is unclear. Medical therapy should be considered in all patients with fluctuations. Treatment should be step-wise and progress from topical therapy to short-term systemic therapy to surgery. Surgery should be followed by topical therapy to prevent recurrence.

13

Familial Patterns of Inheritance of Specific Anosmia to 5α-androst-16-en-3-one (Androstenone). CHARLES J. WYSOCKI, GARY K. BEAUCHAMP, KATHLEEN M. DORRIES* (Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA)

Nearly 40-50% of adults do not perceive an odor after sniffing androstenone. Variation in the ability to smell this compound has a significant genetic component. We previously reported that detection thresholds were more similar among identical compared with fraternal twins, and concordance for the ability to smell the compound was considerably higher among identical than fraternal twins. This earlier work neither determined the mode of inheritance nor provided evidence for a single or major gene effect. To these ends, using methods similar to those employed in testing twins, we obtained detection thresholds for androstenone from both parents and at least two biological offspring from more than 70 families in the metropolitan Philadelphia area (sensitivity to pyridine also was determined). Although strong evidence for underlying genetic modulation of sensitivity to androstenone was observed, patterns of inheritance were complex, suggesting polygenic influence. Additional computer-assisted analyses are on-going and will allow us to determine whether major gene effects can be reported.

*Presently at Department of Psychology, Cornell University, Ithaca, NY. Supported in part by NIH Grant NS 22014 to CJW and the Monell-Jefferson Clinical Taste and Smell Center (NIH NS19616).

14

Oral Sensory and Olfactory Function in Sjogren's Syndrome. J.M. WEIFFENBACH, J. LARSON, A. MACYNSKI, J. ATKINSON, P. FOX and C.TYLENDA (National Institutes of Health)

Sjogren's Syndrome (SS) is an autoimmune disorder affecting salivary and lacrimal glands. SS patients usually complain of eye and mouth dryness. They may also complain of reduced taste and smell acuity. In a previous study, we documented a pattern of taste detection threshold deficits differing markedly from that associated with aging. In the present study, we compare 15 SS patients to 87 unaffected individuals with respect to taste, smell and dryness complaints as well as olfactory recognition and intensity perception of selected oral stimuli. Sucrose and NaCl solutions were presented at 0, 0.056, 0.18, 0.56 and 1.8 M. Temperature was represented by H_2O at 4, 25, 30, 40 and 50 ^{0}C Pressure was elicited by filaments applied to the dorsal tongue to produce a force of 0.0028, 0.408, 1.494, 5.5 and 15 grams. Viscosity stimuli were 1, 40, 400 and 4000 cps solutions of methycellulose. Stimuli were presented in blocks, i.e. all taste then all temperature, pressure and viscosity, with each level of stimulus strength presented twice in a random order. Responses were obtained by cross-modal matching of intensity to distance. Olfactory identification was assessed with a standardized 40 item smell identification test (SIT). More patients than controls complain of diminished acuity for taste(36 vs 6%) and smell (23 vs 7%) and of oral dryness (93 vs 27 %). Patients give larger responses to temperature than do controls. Analogous differences were not observed for taste, pressure or viscosity stimuli. Patient olfactory performance was poorer than that of controls (40 vs 14 below the 50th %tile). Subjective complaints of SS patients occur in a context of altered sensitivity to olfactory and oral thermal stimuli.

15

Spatial Taste Losses Associated With Head Trauma, Upper Respiratory Infection, and Nasal Symptoms LINDA M. BARTOSHUK (Yale University School of Medicine), FRANK A. CATALANOTTO (University of Medicine and Dentistry in New Jersey), APRIL E. SCOTT (University of Connecticut Health Center), and GINA M. SOLOMON (Yale University School of Medicine)

The CCCRC (Connecticut Chemosensory Clinical Research Center) spatial taste test was administered to 94 patients with the following etiologies: head trauma (N=32), upper respiratory infection (N=38), and nasal symptoms (N=24) as well as to 211 control subjects. The test consisted of responses to strong concentrations of NaCl, sucrose, citric acid, and quinine hydrochloride "painted" onto the tongue with sterile, long-handled Q-tips. The areas stimulated were the right and left sides of the front of the tongue, the rear edges of the tongue, and the palate. Subjects judged the intensities using a 9-point scale, with 1="very weak," 5="medium," and 9="very strong." At the end of the test, subjects were asked to judge the intensity produced by swallowing a small amount of each stimulus after moving it throughout the mouth. In all three patient groups, at least some responses were statistically significantly lower than the controls. Data were analyzed as percentages of the control values. Responses from the head trauma patients were the lowest overall and showed equivalent percentage losses for all areas and stimuli. Responses from the nasal symptom patients were the highest overall but responses to sucrose, citric acid, and quinine showed losses specific to the palate. Responses from the upper respiratory infection patients showed effects of both area and stimulus quality. Responses on the front of the tongue tended to show the greatest percent reductions. For all three etiological groups, responses to the swallowed stimuli showed the smallest reductions. This underlines the importance of localized taste testing. When subjects can use their whole mouths to taste, localized losses often go unnoticed.

Supported by Grant NS 16993 from NINCDS.

16

Elemental Analysis of Lymph from the Olfactory Sensilla of the Lobster. R. A. GLEESON, H. C. ALDRICH, H. G. TRAPIDO-ROSENTHAL, and W. E. S. CARR. (The Whitney Laboratory, University of Florida, St. Augustine, FL 32086).

The fluid medium bathing the dendritic extensions of olfactory receptor cells is the environment in which receptor and perireceptor events occur, and its composition can importantly influence these processes [Getchell et al., Prog. Neurobiol. 23: 317-345 (1984)]. Each of the hair-like sensilla of the lobster olfactory organ contains the outer dendritic segments of over 300 sensory neurons; as in the insect, these dendritic extensions are bathed in a sensillar lymph. Unlike the insect, there are no distinct pores in the cuticle of the lobster sensillum for odorant access; instead, the cuticle, which has a "spongy" appearance, is presumed to be freely permeable to odorants in seawater [Grünert and Ache, Cell Tissue Res. 251: 95-103 (1988)]. Manipulating the ionic composition of the seawater bathing the olfactory sensilla has revealed profound effects on the physiological responses of olfactory cells (e.g., ATP-sensitive cells) and on perireceptor biochemical processes associated with the olfactory sensilla (e.g., ectonucleotidase activity and amino acid uptake systems). As an initial step towards defining the composition of the sensillar lymph and its relationship to the permeability of the cuticle, we have utilized energy dispersive X-ray analysis to examine lymph samples expressed from lobster sensilla. Elemental analyses of these samples compared to seawater and hemolymph suggest that the composition of the sensillar lymph is actively regulated.

Supported by NSF grant #BNS-8607513.

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Amino Acid Uptake by the Olfactory Organ of the Spiny Lobster. H.G. TRAPIDO-ROSENTHAL, S. WACHOCKI, M. OTTO, and W.E.S. CARR. Whitney Laboratory, University of Florida, St. Augustine, Florida.

The olfactory organ of the spiny lobster, Panulirus argus, consists of a dense array of aesthetasc sensilla on the lateral branch of the antennule. These sensilla contain primary chemosensory neurons that are in intimate contact with seawater and its low molecular weight solutes. Among these neurons are those that respond to either glutamate or taurine. The studies we report here show that cells within aesthetasc sensilla have: (a) high internal concentrations of these two amino acids; and (b) transport systems capable of internalizing these compounds against the high concentration gradients that exist between cytoplasm and seawater. Each amino acid is transported by a separate, highly specific, sodium-dependent, uptake system. Uptake rates are half-maximal at micromolar concentrations of extracellular amino acids. Studies with competitors for the uptake of taurine demonstrate that: (a) some taurine leaks out of sensillar cells into the receptor environment and (b) the taurine uptake system can reinternalize a substantial proportion of this leakage. We conclude that in the aesthetasc sensilla of P. argus, amino acid uptake systems may serve as a mechanism for maintaining the sensitivity of receptor cells to amino acids from exogenous sources.

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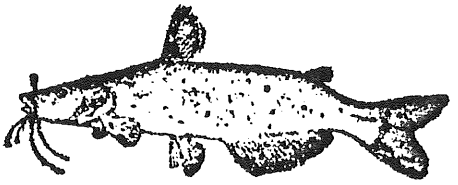
#18 Withdrawn



19

Modulation of K^+ Channels in Mudpuppy Taste Receptor Cells by Intracellular Factors and Taste Stimuli. THOMAS A. CUMMINGS AND SUE C. KINNAMON (Department of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523 and Rocky Mountain Taste and Smell Center, Denver, CO 80262.)

Previous studies have shown that the voltage-dependent K^+ conductance is restricted to the apical membrane in mudpuppy taste cells (Kinnamon, Dionne and Beam, Proc. Natl. Acad. Sci. 85:7023-7027), and that a variety of different K^+ channels make up this conductance (Kinnamon, Soc. Neurosci. Abstr. 14:1062). In this study we have used single-channel recording techniques to characterize further the properties of the different K^+ channels in order to determine their possible role in taste transduction or taste modulation. The most commonly encountered K^+ channels on the apical membrane had conductance values of approximately 65,100,130,175 and 200 pS. All apical K^+ channels showed a small probability of opening at the resting potential in cell-attached patches. The smaller channels (65 and 100 pS) showed a greatly increased probability of opening with depolarization, whereas the larger channels (130-200 pS) required large depolarizations from rest for activation. Inside-out patches revealed that these larger channels were Ca^{++}-dependent as well as voltage-dependent. When inside-out patches containing the smaller channels were excised, the probability of opening steadily increased with time until the channels were open almost continuously; this effect was independent of Ca^{++} concentration. Superfusion with 5 mM ATP restored normal channel activity in some patches, suggesting that these channels are regulated by intracellular levels of ATP. Outside-out patches were used to determine if taste stimuli modulate any of the K^+ channels directly; the 100 pS channel was reversibly blocked by 0.1 mM citric acid, a sour taste stimulus. Experiments are in progress to determine the effects of other nucleotides and taste stimuli on the different K^+ channels.

Supported by NIH grants NS20382 and NS20486-04.

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Selective Interactions of Lectins with Amino Acid Taste Receptor Sites of the Channel Catfish. D. LYNN KALINOSKI , LOIS C. JOHNSON and JOSEPH G. BRAND (Monell Chemical Senses Center, 3500 Market St. and Veterans Administration Medical Center, Philadelphia, PA 19104, USA)

Previous studies of lectin interactions with chemosensory receptors of the channel catfish had demonstrated that PNA selectively inhibited the binding of the stimulus L-arginine but not L-alanine to olfactory receptors sites (Kalinoski et al., Brain Res. 1987). The lectins ConA and WGA inhibited the binding of both stimuli to olfactory receptor membranes. None of these three lectins inhibited the binding of either stimulus to taste receptor sites. We have extended the study of lectin interactions with chemosensory receptors to five additional lectins of differing carbohydrate specificities, phaseolus vulgaris (PHA), dolichos biflorus (DBA), ricinus communis (RCA I), pisum sativum (PSA) and Jacalin. When the unconjugated lectins were preincubated with Fraction P2 from taste epithelium at a concentration of 50 ug/ml, a significant inhibition of L-[^{3}H]-arginine binding by the lectins PHA and RCA I (68% and 74%, respectively) was observed. These two lectins only minimally inhibited the binding of L-[^{3}H]-alanine to Fraction P2 (3% and 25%, respectively). Furthermore the lectin DBA appeared to be moderately selective for the inhibition of L-[^{3}H]-alanine over L-[^{3}H]-arginine binding to taste membrane fractions (28% to 8%, respectively) while Jacalin moderately inhibited the binding of both L-alanine and L-arginine to Fraction P2. Inhibition of taste receptor binding by all lectins was time- and dose-dependent, and was fully inhibited by incubation in presence of the appropriate hapten sugar. These results indicate that there is a differential action of lectins upon the two taste receptor sites responsible for recognizing L-alanine and L-arginine and further suggest that lectins may prove useful tools in the purification of taste receptor proteins.

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Membrane Proteins Unique to Olfactory Cilia Identified by Monoclonal Antibodies. ANN E. PETRO, ANN M. RIVERS and ROBERT R. H. ANHOLT (Department of Neurobiology, Duke University Medical Center, Durham, NC 27710).*

We raised monoclonal antibodies (mAbs) against chemosensory cilia from the olfactory epithelium of Rana catesbeiana. Of 36 mAbs produced, 19 reacted with Western blots. Three patterns of immunoreactivity were evident: 1) antibodies that visualize one distinct band; 2) antibodies that visualize multiple distinct bands, reflecting proteolysis; and, 3) antibodies that stain one or more diffuse bands, reactive with carbohydrate groups. Four mAbs reacted only with olfactory tissue and not with membranes from brain, heart, liver, kidney and lung. In olfactory cilia, mAbs 26 and 42 both stain diffuse, but distinct, regions around 62 kDa and 125 kDa. The 125 kDa region is not observed with membranes prepared from olfactory nerve. mAb 8 visualizes a 59 kDa band in olfactory cilia and does not react with olfactory nerve membranes. Comparing the staining patterns of mAb 8 obtained under reducing versus non-reducing conditions indicates that the antigen recognized exists as a dimer. Deglycosylation of olfactory cilia by endoglycosidase H shows that mAbs 8, 26 and 42 recognize carbohydrate moieties. A fourth mAb, mAb 34, identifies an 87 kDa polypeptide unique to olfactory cilia. Immunoreactivity of this mAb is not affected by deglycosylation or reduction. These studies show that olfactory cilia contain membrane glycoproteins with unique carbohydrate moieties. Moreover, proteins unique to olfactory cilia, identified by these mAbs, are likely to play important roles in odorant recognition and/or olfactory transduction.

* Supported by Army Research Office grant DAAL03-86-K-0130 and NIH grant NS24521.

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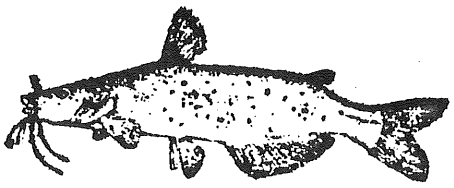
#22 Withdrawn



23

Three-Dimensional Models of the Glucose Receptor Sites of Gustatory Cells of the Tobacco Hornworm, Manduca sexta. JAMES L. FRAZIER (E. I. Du Pont de Nemours) and PATRICK YUK-SUN LAM (E. I. Du Pont de Nemours)

Three-dimensional models of the glucose receptor sites in the taste cells of the styloconica of Manduca sexta caterpillars were developed by recording responses of individual taste cells to synthesized and commercially available epimers, deoxy-, and fluro-substituted glucose derivatives at each carbon position. These models indicate the receptor site hydrogen bond donors and acceptors for the C-1,C-2,C-3, C-4, and C-6 positions of the glucose stimulus. In both the lateral and medial styloconic taste cells, all the hydroxyl groups of glucose, except that of C-1, are involved in binding via hydrogen bond donation. The medial site is more specific than the lateral site, with the C-2 hydroxy required for binding. In addition the C-1 and C-3 oxygens are involved as hydrogen-bond acceptors for the medial site. The differences in the lateral and medial receptor site models were independently confirmed by synthesis of cyclic guanidines. The cyclic guanidine without oxygens to accept hydrogen bonds at the C-1 and C-3 positions failed to stimulate the medial site, while stimulating the lateral site nearly as well as glucose.

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The Cyclic AMP Chemoreceptor of Paramecium. I.M. SCHLICHTHERLE, B. COTE, J. ZHANG, J. L. VAN HOUTEN (University of Vermont, Burlington, VT 05405).

A cell surface receptor for cyclic AMP has been identified as a doublet of membrane proteins of Paramecium tetraurelia. This was first identified by affinity chromatography, using cAMP-agarose. The proteins specifically elute with cAMP and 5'AMP (a partial antagonist of cAMP-induced chemoresponse behavior), but not with cGMP or 5'GMP. The proteins have a molecular weight of about 48,000 on SDS PAGE. The protein has an unusually acidic pI (2.5) and carbohydrate recognized by wheat germ agglutinin. Antibodies have been produced against gel slices containing the cAMP receptor doublet. These antibodies very selectively eliminate the cells' behavioral response to cAMP, while they have no effect on responses to other stimuli (lactate and ammonium) or little effect (acetate). The latter is of interest because elution of the affinity column with acetate brings off a small fraction of the doublet. The antibodies are being used in Western blots to examine the specificity of the anti-serum for the 48 kd proteins among membrane proteins. Western blots have shown that the 48 kd proteins are not identical either to the regulatory subunit of the cAMP-dependent protein kinase of Paramecium, or to the cAMP receptor of similar molecular weight from Dictyostelium discoideum. CNBr generated fragments of the protein are being prepared for microsequencing. Oligonucleotide probes made from an inferred DNA sequence and the polyclonal antibodies will be used to screen a λ gt11 library of Paramecium genomic DNA. The λ gt11 library of Paramecium DNA has been prepared and being assessed for insert size.

Supported by NSF and the VRCC.

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Calcium ATPase as Part of the Transduction Pathway in Paramecium Chemoreception. M. V. WRIGHT and J. VAN HOUTEN (University of Vermont, Burlington, VT 05405).

Paramecia are unicellular ciliated organisms that can detect specific chemicals in their environment, probably as food cues, and accumulate in areas of optimal concentration. The primary response of the cell to an attractant stimulus (e.g. folic acid) is a plasma membrane hyperpolarization. This change in membrane potential can be measured as a 0.2 nA outward current, but it has been difficult to directly determine the ionic species carrying the current. The hyperpolarization is not voltage-dependent and is not affected by external K or Na. However, increasing evidence suggests that a Ca^{2+} extrusion pump may be involved. Here we present some of that evidence: Paramecia are normally attracted to folic acid in behavioral T-maze assays. A mutant (K-shy, courtesy of T. Evans) thought to be defective in Ca^{2+} pumping is not attracted under identical conditions. Lithium treatment of wild type causes the repulsion of the cells from folate, normally an attractant. Further investigation reveals that Li also inhibits the normal rate of Ca^{2+} efflux. We have isolated a Mg^{++}-dependent Ca^{2+}-stimulated ATPase in the cell body membranes of Paramecium. This ATPase exhibits all the characteristics of a pump. Addition of Li to the assay inhibits CA^{2+}ATPase activity by 15-20%. Assuming a coupling ratio of 1-2 Ca^{2+} transported/ATP hydrolyzed and that this ATPase is the major contributor to Ca^{2+} homeostasis, this inhibition could account for the 0.2 nA current.

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Chronic Recordings from Implanted Electrodes in the Rat Glossopharyngeal Nerve. ROBERT M. BRADLEY AND ROBERT D. SWEAZEY, Dept. Biologic and Materials Sciences, University of Michigan.

Most information on the response properties of gustatory receptors is based on recordings from acute preparations of anesthetized animals. However, the taste bud cells are replaced throughout the life of the animal, and it is not clear from acute electrophysiological experiments if the response characteristics of single taste axons change as the innervated receptors turn over. To examine the long term stability of the response of gustatory fibers, we are developing an implantable electrode to make chronic recordings from the glossopharyngeal nerve. When the cut ends of the glossopharyngeal nerve are placed in a short length of small diameter silastic tubing, the nerve regenerates through the tube and stimulation of the posterior tongue with chemical, mechanical and thermal stimuli results in neural responses that can be recorded central to the cut after 30 days. Furthermore, it is possible to record multiunit taste responses after 30 days regeneration time from a small diameter wire electrode placed in the tubing. Currently we are experimenting with electrode arrays that consist of small diameter holes etched in silicon wafers to determine the feasibility of chronic recordings from few or single fibers in the glossopharyngeal nerve.

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Identification of Action Potential Responses from Individual Fly Receptor Cells by Fourier Analysis. C.N. DESOURDY AND L.M. KENNEDY (Clark Univ., Worcester MA 01610).

Digitization of analog neurophysiological data using currently available computer hardware is straightforward, but analysis of converted multiunit responses can be difficult. Methods involving discrimination by amplitude differences of action potentials from various cells are sufficient in systems where the amplitudes differ significantly from each other. Further discrimination is possible using slopes, peak-to-trough parameters and template-matching techniques (Frazier & Hanson, in Insect/Plant Interactions, Miller & Miller (eds.) Springer-Verlag, 1986). However, the amplitudes and/or shapes of action potentials may change with firing over time (Fujishiro et al., J. Insect Physiol. 30, 1984, 317; M. Simmonds, presented at XVIIth Internatl. Chemorecept. Workshop on Insects, 1987), with pharmacological treatment, or with intermittent stimulation (Kolodny & Kennedy, Chem. Senses 13, 1988, 545). These changes make discrimination of responses from individual cells more difficult.

To solve this problem, we are using the Fourier Transform to characterize and recognize the potentials from individual fly taste receptor cells. First we identify all the action potentials in the digitized record, using slope and time course parameters. A Fourier Transform is then run on each action potential. The power spectra, partial power and relative power parameters thus obtained differ for responses from individual cells. When changes in action potential amplitudes and/or shapes occur, evaluation of resultant changes of the power spectra should allow identification of cells of origin for the changed responses. At present, we find some variability in the power spectra of responses from a given cell due to the method by which the potentials are selected. Improved recording amplifiers and selection algorithms are expected to minimize this variance.

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28

Antagonism of the Gerbil's Sweetener Response by Copper Chloride. Latchman Somenerain and William Jakinovich Jr. (Department of Biological Sciences, Herbert H. Lehman College and the Graduate School, City University of New York, Bronx, NY 10468, USA)

It has been shown by Iwasaki and Sato that the mouse's chorda tympani nerve response to sweeteners is differentially suppressed by $CuCl_2$. In particular, this compound suppresses taste responses to sucrose, fructose, glucose, maltose and saccharin but had hardly any effect on the taste responses to the sweet amino acids glycine, L-alanine, L-serine, L-proline, L-valine and D-tryptophan. The purpose of these studies was to determine if the $CuCl_2$ antagonism is a species specific effect, and if not, to test its antagonistic properties on additional sweeteners.

In the gerbil, $CuCl_2$ will differentially antagonize the sweetener response, but only if it is applied for longer periods of time than used by Iwasaki and Sato (30 minutes instead of 30 seconds). In our hands responses to the following sweeteners were suppressed; L-proline, L-alanine, D-tryptophan, 6-chloro-D-tryptophan, sucrose, maltose, lactose, tetrachloro-galacto-sucrose, glucose, fructose, methyl α-D-glucopyranoside, glycerol, sorbitol, saccharin, L-4'-cyano-3'-(2-2-2-trifluoro) acetamido-succinanilic acid, phenethyurea, and stevioside. The taste responses of the sweet amino acids L-serine and glycine were not effected by $CuCl_2$. Also, taste responses to NaCl were not effected.

The significance of the work is that it indicates that there are additional sweet-taste receptors on the gerbil's taste receptor cell membrane; a sugar receptor which interacts with sugars and most other sweeteners including some sweet amino acids, and one which interacts with glycine and L-serine.

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Electrophysiological Studies of S-fiber Specific Taste Stimuli in the Hamster Chorda Tympani Nerve. WALTER E. MYERS, THOMAS P. HETTINGER & MARION E. FRANK (Department of Biostructure & Function, University of Connecticut Health Center, Farmington, CT 06032)

A number of structurally heterogeneous compounds are known to specifically activate hamster chorda tympani S-fibers. These compounds are perceived by hamsters as sucrose-like in conditioned aversion studies. Whether the recognition of these compounds is mediated by a single "broadly-tuned" recognition site or by multiple recognition sites, is not known. Sucrose, a prototypic S-fiber stimulant, when applied to the anterior tongue, will induce a chorda tympani response that decays with time. Subsequent applications of the same concentration of sucrose will not induce any further neural response unless the tongue first receives a distilled water rinse. This phenomenon is known as self-adaptation. Self-adaptation is also common to other S-fiber stimuli. If all S-fiber stimuli interact with the same recognition site, it would be expected that full cross-adaptation would be seen when saturating concentrations are used as adapting agents and test stimuli. We addressed the issue of the nature of S-stimulus recognition by reciprocal cross-adaptation of the hamster chorda tympani response using saturating concentrations of sucrose (.56M), glycine (.56M), and calcium cyclamate (.032M) as determined by dose-response studies. Results of these experiments show that following complete self adaptation by each of the compounds, the chorda tympani nerve responses to the test compounds were not greatly reduced (< 30% reduction) as compared to control responses (responses prior to adaptation). The relatively small degree of cross-adaptation between these stimuli cannot be explained by a simple one- site model of S-fiber activation.

Supported by NIH grant NS 16993.

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Sugar Response Characteristics in the Rat Chorda Tympani Nerve During Development. SHUITSU HARADA (University of Cincinnati College of Medicine), SHINJI MAEDA, and YASUO KASAHARA (Kagoshima University Dental School)

Integrated taste responses to various sugars were studied in the rat chorda tympani nerve during development. Animals were male Sprague-Dawley rats of 1, 2, 3, 4, or 8 weeks of postnatal age. Sugar stimuli were concentration series of sucrose, fructose, lactose, maltose, glucose and galactose. When compared with 0.1 M NH_4Cl, the relative response magnitudes for these sugars at 0.5 M increased from 1 to 2 weeks of age and gradually decreased with age beyond 2 weeks. During 1 to 4 weeks of age, the order of response magnitude (at 0.5 M) was sucrose > lactose > fructose > = maltose > = glucose > = galactose. However, at 8 weeks of age, lactose was less stimulatory than fructose. The off-responses observed at the initiation of the distilled water rinse following sugar stimulation were larger and more frequent in young than in adult rats. Although there was no correlation between on- and off-responses for any 0.5 M sugar in 2-week-oid rats, highly negative correlations between sugar responses and off-responses were obtained for 0.5 M sucrose (r = -0.931, n = 19) and for 0.5 M lactose (r = -0.859, n = 10) in 8-week-old rats. In a cross-adaptation experiment, different sugars did not cross-adapt well in either young (30 - 40% suppression) or adult (20 - 30% suppression) rats. These results suggest that the receptor mechanisms for these sugars are not the same and that they become somewhat more specific with maturation. When the tongue of 2-week-old rats was treated with 2% pronase-E for four minutes, responses to sugars decreased by about 50% and reversibly recovered within one hour. However, in 8-week-old rats, even a three-minute application of pronase-E produced much stronger depression, with no apparent recovery within an hour after treatment. These experiments suggest that processing of gustatory information for sugars in the rat peripheral nervous system changes during development.

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Gustatory Responses of the Channel Catfish, *Ictalurus punctatus*, to Nucleotides and Related Substances. J. LITTLETON, J. KOHBARA, W. MICHEL and J. CAPRIO. (Department of Zoology and Physiology, Louisiana State Univ., Baton Rouge).*

In a previous report (Michel et al., AChemS IX, 1987), nucleotides and related substances (NRS) were shown to be effective stimuli for the facial taste system of the channel catfish, *Ictalurus punctatus*. The detection thresholds for the more effective NRS were estimated at 10^{-4} M and NRS receptor site types independent of those for L-alanine and L-arginine were indicated. In the present study, the number of NRS tested was expanded and NRS receptor site types were identified. Although the purine base, adenine, was highly effective, the hypoxanthine-based nucleosides and nucleotides were generally more stimulatory than the corresponding adenine-based substances in multiunit preparations. Unique receptor site types for adenine, adenosine, inosine, AMP and ATP were identified through cross-adaptation experiments. The identified NRS receptor site types correspond to the by-products of purine metabolism found in most animals. For individual taste units, the majority of the chemically-sensitive neurons responded primarily to amino acids. Although a few facial neurons were encountered that were only/primarily responsive to NRS, the majority of the NRS information was carried on gustatory neurons that were also responsive to amino acids. Preliminary evidence indicates that the amino acid taste responses of some fibers, which themselves were not stimulated by AMP, were enhanced by pre-treatment of the gustatory receptive field with AMP. Based on the relatively high sensitivity of the facial taste system to amino acids and a lower sensitivity to NRS, amino acids are possibly more important than NRS in the localization of food from a distance. Presumably, NRS information is important upon initial contact, when the quality of the food is determined.

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Gustatory Response Latencies of the Frog. STEVEN T. KELLING, BRUCE P. HALPERN (Cornell Univ. Physiology/Psychology/NBB. Ithaca NY 14853-7601)

Gustatory neural response latency (RL), the time between stimulus arrival on the tongue and changed afferent activity, is typically 20-150 ms in vertebrates. However it has been reported that RL for the Bullfrog were >500 ms for several stimuli (Sato, et al., Brain Res.,1988). We show that RL for the Bullfrog are equal to other vertebrates. METHODS Whole glossopharyngeal nerve recordings were made on 5 bullfrogs (Rana Catesbiana). Stimulation of the tongue was with automated pipettes delivering 10 microliters to the same tongue region. One pipette presented the control stimulus (CS); the other, the stimuli (S): 2 mM QHCl (QH), 10 mM $CaCl_2$ (Ca), Distilled Water (DW), $1.3x10^{-5}$ (C5), $1.3x10^{-6}$ (C6), and $1.3x10^{-7}$ (C7) Cantharidin. A second control was the activation of a pipette with no stimulus presented (NS). Ringers was the CS and the adapting solution, and the solvent for all S. There was a 3 minute intertrial interval. Every S trial was followed by a CS trial. Each stimulus series was presented 10 times for each frog. Amplified nerve activity was digitally summated in 10 ms binwidths, and A/D converted at 500 Hz for 100 ms prior to stimulus onset and 3000 ms thereafter. RL was determined using the Wilcoxon matched-pairs signed-rank test, comparing the S with its matched CS, and comparing S and CS with NS. The RL was defined as the first bin, of a series of 5 sequential bins, with $p \leq 0.05$ when comparing S with both CS and NS trials. RESULTS RL were: QH-100 ms, Ca-70 ms, DW-70 ms, C5-110 ms, C6-90 ms, and C7-not obtained. For all stimulus series, CS did not reach RL criteria when compared to NS trials. Conclusions When stimulus presentation techniques avoid large mechanoreceptor responses from the frog tongue, gustatory response latencies are similar to those found in other vertebrates.

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Temporal Profile of the Responses of Moth Olfactory Receptor Neurons to Pheromone Components. P.F.BORRONI and R.J.O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA).

Natural chemical stimuli seldom occur as isolated pulses of single compounds. Most commonly chemosensory neurons must manage trains of stimuli of various temporal patterns and complex combinations of concentration and composition. The temporal adaptation profile of these neurons plays a critical role in the process of extracting information from complex stimuli. With the present experiments we are beginning a study of the temporal parameters of chemosensory responses of insect antennal receptor neurons. Extracellular recordings from single olfactory neurons are obtained by inserting a tungsten microelectrode through the cuticle at the base of sensilla trichodea of male *Trichoplusia ni*. Stimulus delivery through computer controlled valves insures a relatively rectangular stimulus profile. Duration-response functions to various identified pheromone components are obtained, using pheromone dosages of 0.0001, 0.001, and 0.01 $\mu g/\mu l$, and the following stimulus durations: 0.1, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 3.0, 5.0, 10.0, 20.0 and 40.0 seconds. The relationship between stimulus duration and response magnitude appears to be linear for most of the duration range tested. The response to the onset of stimulation shows a phasic-tonic time course; however, the tonic portion does not adapt, even for the longest stimulus durations. To determine whether and how the sensitivity of receptor neurons varies during a long stimulus pulse, short duration stimuli (0.1s) are pulsed at various times during stimuli of longer duration.

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Response Dynamics of Lobster Olfactory Neurons During Simulated Natural Sampling. H-P. MARSCHALL (Alfred-Wegener Institute, Bremerhaven, FRG) and B.W. ACHE (Whitney Laboratory and Depts. of Zoology and Neuroscience, Univ. of Florida)

Early events in chemoreception should have dynamic properties that are consistent with those of the output of the receptor cell. The response dynamics of olfactory receptor cells are difficult to define, however, because (1) the discharge closely follows the stimulus profile and (2) the stimulus profile is typically shaped by dynamic events (e.g., sniffing) that are hard to reproduce experimentally. In lobsters, stimulus access to the olfactory (aesthetasc) receptors is gated by an intermittent sampling reflex called "flicking". Using an olfactometer designed to mimic flicking, we characterized temporal parameters of the discharge of lobster olfactory receptor cells. Sections of the olfactory organ (antennule) were presented with 30 to 60 odor pulses of 0.1 to 0.5 sec duration at 0.5 to 0.1 Hz over a range of concentrations. The response latency was brief and varied inversely with concentration, typically from 50 to 400 msec. A few cells had latencies in excess of 1.5 sec. The response was strongly phaso-tonic; concentration-dependent maximum instantaneous frequencies up to 80 Hz were reached immediately and decayed within 100 msec to a plateau frequency 10-30% of maximum. The plateau frequency saturated at lower concentrations than did the maximum frequency. The response duration varied directly with concentration, typically from 100 to 1500 ms. Responses that terminated within the sampling interval showed no long term adaptation at sampling rates of at least 0.5 Hz.

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Effects of high ammonium background on stimulus-response function of narrowly tuned chemoreceptor cells. RAINER VOIGT, CARA COBURN and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543).

Ammonium (NH_4) is a biologically relevant stimulus and high levels are found in coastal waters. Lobster have narrowly tuned NH_4 receptors. We tested the effects of an elevated NH_4 background on the stimulus-response (SR) functions of 22 hydroxyproline-(Hyp), 6 arginine-(Arg), and 4 taurine-(Tau) sensitive chemoreceptor cells of the lobster's (Homarus americanus) medial antennules. Responses of single receptor cells were recorded extracellularly using a suction electrode. After identification of a single cell with a search stimulus (equimolar mixture of Hyp, Arg and Tau; each at $7x10^{-5}$ M) individual responses to the mixture components were measured to identify the best stimulus. Then, SR functions were determined in artificial seawater (ASW) for the best-stimulus and NH_4 ($7x10^{-9}$ to $7x10^{-3}$ M). After a 3-min adaptation to a 10^{-4} M NH_4 background, the SR functions were re-determined. Finally, as a control for viability and NH_4 effects, SR functions were repeated in ASW. Of 22 narrowly tuned Hyp-cells only two responded slightly to high concentrations of NH_4 in ASW. None of the 22 cells altered SR functions in the NH_4 background. Of 6 Arg cells 4 responded equally to Arg and Hyp; three of these cells gave small responses to high NH_4 stimuli in ASW; the two remaining cells were narrowly tuned to Arg. None of these 6 cells was affected by the NH_4 background. Two of four narrowly tuned Tau cells gave strong responses to NH_4 in ASW; one of these two cells showed suppressed responses in the NH_4 background. We conclude that narrowly tuned Hyp and Arg cells on the medial antennule of the lobster are unaffected by even abnormally high NH_4 backgrounds. This characteristic makes them suitable for detection of chemical contrast in high noise environments.

Supported by NSF grant BNS 8812952 to JA

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Self- and Cross-Adaptation in Chemoreceptor Cells H.F.GERARDO, RAINER VOIGT and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543).

To understand the filter function of receptor cells it is important to know their adaptation properties. We recognize self-adaptation where stimulus and adapting background are the same, and cross-adaptation where they are different. We do not deal here with time courses of adaptation or disadaptation. Previous experiments in lobsters (*Homarus americanus*) have shown that self-adaptation results in a parallel shift of stimulus response (SR) functions (Borroni & Atema, 1988) and cross-adaptation results in a change of slope but no shift of SR functions (Borroni & Atema, subm.). These experiments were done separately in different populations of ammonium-best (NH_4) cells of lobster legs. Building on these results we tested a single population of glutamate-best (Glu) cells of lobster legs for the effects of self and cross adaptation. Individual cells were located with Glu and tested for tuning with equimolar Glu, NH_4, Hyp (hydroxyproline) and Bet (betaine) stimuli in ASW (artificial sea water). Then we determined a Glu SR function in ASW, followed by an SR function of a 21-compound mixture (Mix, including Glu) in ASW, followed by an SR function for a (Mix-Glu) mixture in ASW. Then Glu SR functions were determined in backgrounds of Glu, Mix, and Mix-Glu, followed by a viability check. The results allow separation of cross- and self-adaptation effects when tested in the same cells and hence determine further the filter properties of individual cells. The results also test predictions of an earlier receptor model.

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Pheromone catabolism by sensillar aldehyde oxidases in two species of moth. R. RYBCZYNSKI, R. G. VOGT, J. REAGAN and M.R. LERNER (Yale University School of Medicine, New Haven, CT 06510 USA)

If a single odor molecule repeatedly interacts with the receptor apparatus, it will generate a high level of sensory noise. High backround noise will make it difficult for an animal to detect spatial or temporal changes in their olfactory environment. A sensitive olfactory system is very important for the reproduction of moths. One way that moths could decrease sensory noise is to biochemically regulate sex pheromone half-life in olfactory sensilla via enzymatic processes. A male-specific, antenna-specific esterase has been described from the moth *Antheraea polyphemus* which appears to be responsible for regulating the half-life of acetate ester pheromone of this species (Vogt R.G. and L.M.Riddiford (1981) *Nature* 293: 161-163). We now report that two species of moths that produce aldehydic pheromones also contain antenna-specific, pheromone- metabolizing enzymes, namely aldehyde oxidases (AOX). Female *Manduca sexta* produce two aldehydes as sex pheromones: bombykal [(E,Z)-10,12-hexadecadienal] and (E,E,Z)-10,12,14 -hexadecatrienal. The receptor lymph from the sensory hairs of *M. sexta* contains a soluble AOX that efficiently oxidizes aldehydes including bombykal to carboxylic acids (Rybczynski, R., J. Reagan and M.R. Lerner (1989) *J. Neurosci.* in press) (the second pheromone is not yet available for testing). Lower levels of this AOX are also found in sensory hairs from females but it is noteworthy that female *M. sexta* are capable of smelling non-pheromone odors including *trans*-2-hexenal. Female *Antheraea polyphemus* produce an aldehydic pheromone [(E,Z)-6,11 -hexadecadienal] as well as the acetate ester. We have found that male *A. polyphemus* contain a sex- and antennal-specific AOX, in addition to the esterase described above, that efficiently metabolizes aldehydes. This AOX is related to but biochemically distinct from the antennal AOX of *M. sexta*. Using *in vitro* measurements of kinetic parameters we estimate that at physiological concentrations the half-life of bombykal in the pheromone-sensitive sensilla of male *M. sexta* to be less than a millisecond. These data suggest that antennal enzymes are wide spread and that these enzymes play a dynamic role in the initial events of odor reception.

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Chemical Communication in Dolphins: Chemical Constituents of the Perianal Gland. MICHAEL A. ADAMS (Monell Chemical Senses Center, University of Pennsylvania, 3500 Market Street, Phila., PA 19104), PAUL E. NACHTIGALL (Naval Ocean Systems Center, P. O. Box 997, Kailua, HI 96734)

For many mammals, chemical communication is an important means of transferring information among members of a species, and for gathering information about the milieu in which the animal lives. The sea is a particularly rich source of chemical stimuli, and most organisms living in the sea make some use of these chemical stimuli for regulation of their behavior: they are important for prey finding, mate location and pair-bonding, positional or directional orientation, and so forth.

The Atlantic bottlenose dolphin (Tursiops truncatus) is a mammal that has adapted to an exclusively aquatic life; its organ systems have been modified in ways which allow these animals to function well in, what is for mammals, an extreme environment. Although this creature seems to lack any olfactory capacity, it does possess the requisite neuroanatomical structures for gustation. The presence of a well-developed trigeminal nerve lends further anatomical support to behavioral data showing that dolphins possess a chemosensory capability. This capability may perhaps be useful for regulation of behaviors that have been shown, in other mammalian species, to be chemically-mediated, for example, mating, social recognition, food selection, and avoidance of dangerous substances in the environment.

Samples of perianal gland secretions were collected from male dolphins and analyzed by combined gas chromatography-mass spectrometry. The presence of several long-chain organic acids was observed. The identities of these substances and their possible function in the dolphin will be discussed.

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High Resolution Analysis of Odor Signals Paul A. MOORE, GREG GERHARDT* and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543 USA * Department of Psychiatry, University of Colorado Health Sciences Center, Denver, CO)

Odor plumes serve as environmental signals for chemoreceptors. Odor signals are shaped by turbulent dispersal and molecular diffusion. The quantitative measurement of odor signals at the receptor is crucial to the understanding of receptor cell responses and chemically mediated animal behavior. To accurately predict cell responses or animal behaviors, the measurement of odor signals must be made at appropriate time and space scales. These scales may range from 100 µm and 25 ms for receptor structures to milliliters and seconds for signals sampled by whole receptor organs. Much larger scales can be sampled by large animals during behavioral search. With electrochemical probes, we can measure chemical tracers at the small time and space scales of receptor structures. Since the probe measures electron transfer (redox reaction) of tracer chemicals at the probes surface tracer chemicals must strike the exposed surface to be measured. This is spatially and temporally similar to the binding of (aquatic) odorant molecules to receptor proteins. The spatial resolution is limited to the exposed surface area of the probe, which can be modified to suit our needs. We model a lobster olfactory organ. The current time resolution is 5 ms. We will present data on three applications of this system to external chemoreception. 1. The quantitative measurement of odor plumes. 2. On-line stimulus monitoring in electrophysiological recording chambers. 3. Measurements of boundary layers and diffusion barriers around receptor structures.

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Developmental Changes in Larval Abalone Behavior in Response to GABA. L. A. Barlow (Univ. of Washington)

Abalone larvae are induced to settle and metamorphose in the presence of the coralline alga, *Lithothamnium*. The neurotransmitter, GABA, which competes at the level of the receptor with a peptide isolated from the coralline alga, also induces these molluscan veliger larvae to settle and metamorphose (Trapido-Rosenthal and Morse, 1984), once they are developmentally competent (day 7 post-fertilization). Video recordings were used to assess larval responses to GABA exposure. Larvae arrest their swimming cilia in response to a brief exposure to GABA. Both the percentage of larvae arresting their cilia and the mean duration of ciliary arrest increases with larval age and GABA concentration. Early in larval life, prior to the time at which larvae are competent to settle, only high concentrations of GABA (.1-1mM) affect larval swimming activity; whereas after day 10 post-fertilization, well after larvae are competent to settle, a steadily increasing percentage will arrest their cilia in response to 1uM GABA. The effects of GABA exposure on ciliary arrest persist after wash-out and become more pronounced in larvae as they age. In addition, GABA causes an age- and concentration-dependent increase in foot exploratory behavior, which to some degree parallels the ciliary response. The timing of enhanced behavioral sensitivity generally occurs after the larvae have become competent to settle. Both ciliary arrest and foot exploration, resulting in foot attachment, are components of settlement behavior. Settlement and metamorphosis in response to GABA also change with larval age. 10uM GABA causes high levels of settlement within 24 hours in competent larvae regardless age. The timing of subsequent metamorphosis, on the other hand, appears to vary with age. Day 7 larvae metamorphose between 48-72 hours after settlement whereas older larvae (day11-15) metamorphose quickly within hours of initial settlement. It appears as though settlement and metamorphosis become temporally more tightly coupled as competent larvae mature. The relationship between the timing of enhanced behavioral sensitivity to brief exposures to GABA, and changes in the coupling of settlement and metamorphosis will be discussed.

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Ecological constraints on physiological responses of Hydroxyproline sensitive chemoreceptor cells. CARL MERRILL and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543 USA)

Hydroxyproline-rich compounds released from the degradation of tissues containing collagenic components may play an important role in lobster chemoreception. A significant population of chemoreceptor cells of the lateral antennules of the lobster, *Homarus americanus*, is narrowly tuned to trans-4-hydroxy-L-proline (Hyp), suggesting that Hyp may be an important chemical signal for this organism. The free amino acid by itself does not evoke a known behavioral response from the lobster perhaps because it carries meaningful information only as a component of a specific mixture of free amino acids and other compounds or as part of a specific peptide. Gelatin, denatured collagen, is rich in Hyp residues (14 % bound Hyp) and elicits a physiological response from many Hyp sensitive cells, sometimes even greater than the response to Hyp. The low firing rate of Hyp sensitive cells (mean response to 10^{-5} M is 9 spikes/500 msec, n = 24 cells) and the response to gelatin solutions and the high molecular weight fractions of gelatin solutions (>1KD and >12 KD) suggest that these cells may be tuned to another "best" stimulus, perhaps an oligopeptide rich in Hyp residues. In this study, oligopeptides containing Hyp residues are used as test stimuli. Responses to these stimuli are compared to responses to equimolar mixtures of free amino acids that are components of these peptides. Ecologically relevant sources of Hyp-rich peptides including mussel, *Mytilus edulis*, byssus threads and lobster molt shells also serve as a source of test stimuli. Mussels, an important prey species of the lobster, periodically degrade their Hyp-rich byssus. Degradation of connective tissue during molting in crustaceans may also release pulses of Hyp and Hyp-rich compounds that may contain information of great importance to lobsters in monitoring the molt status of conspecifics. Similar compounds may also indicate food quality, i.e. food decay state.

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Microgradients, Microbes and Random Walks.
M. LEVANDOWSKY (Haskins Labs, Pace U., N.Y., NY 10038)

The basic microbial model of biased random walk chemosensory response is examined in several concrete examples, and several spatio-temporal scales. At the smallest scale, for very minute (less than 1 micron) bacteria it is claimed that a chemosensory response of the usual sort would not be adaptive. At the next scale, typical of many bacteria and smaller protists, a chemokinesis (sensu latu) rather than a chemotaxis is appropriate. Here the distinction between spatio-structural gradients and absolute levels becomes critical. The next scale consists of larger protists and smaller invertebrates, many of which have a swimming Reynolds number of the order of one. For these organisms a true chemotaxis can be appropriate.

Implications of these various constraints for physiological mechanisms are discussed. In the case of the biased random walk type models, different distributions of excursion lengths between changes of direction have implications regarding the presence or absence of a sort of 'memory' in the organism.

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Peptide Attractants of Hermit Crabs. R. HUFF, C. KRATT, and D. RITTSCHOF (Duke University Marine Laboratory, Pivers Island, Beaufort, NC USA).

Hermit crabs are known to respond to chemical cues that signal the presence of shells that they occupy. Experiments were performed to further clarify the nature of these chemical cues. Three approaches were taken: 1) attractant cues generated by trypsin digestion of snail muscle were partially characterized; 2) major snail proteins were purified and tested for cue generation by treatment with trypsin; 3) several known peptides and high (millimolar) concentrations of binary mixtures of amino acids were tested for crab attractant properties. Bioassays were performed in the field.

Peptides <500 D generated by trypsin digestion of snail muscle were potent and specific attractants of crabs. Of the peptides that were less than 500 D, those with low affinity for C18 silica were most potent. Snail proteins tested as sources of peptides were hemocyanin, a mixture of hemolymph proteins after removal of hemocyanin, and snail myosin and actinomyosin. Digestion of hemocyanin with trypsin resulted in low levels of response. Digestion of both myosin and actinomyosin yielded potent attractant peptides. Actinomyosin was the source of the most potent peptides. Digestion of trypsinogen with enterokinase yields only the hexapeptide Val-Asp-Asp-Asp-Asp-Lys and this preparation was as potent in attracting one species of hermit crab as digested snail flesh. The specificity of attractions appears to be based upon the protein substrates. Supported by ONR N00014-86-K-0261 and ONR N00014-87-J-1267.

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The Feasibility of Chemo-orientation in Complex Natural Habitats. RICHARD K. ZIMMER-FAUST and SALLY MACINTYRE (Marine Environmental Sciences Consortium, Dauphin Island, AL 36528, and Marine Science Institute, University of California, Santa Barbara, CA 93106).

Because odor plumes are filamentous in nature, models that rely on time-averaged estimates of odor concentration often fail to predict elicited animal response. It is difficult to measure odor distributions over time and space scales relevant to chemosensing by individual organisms. We recently developed an instrument useful in determining the dispersal of fluorescent dye as a tracer of waterborne scent in an aquatic environment. The instrument allows measurements to be made of chemical gradients over spaces naturally separating the bilateral olfactory organs of small benthic organisms. Our initial field experiments showed that the transport of dye was patchy in time and space, even though it was released continuously from a single point source. In fact, the lifetime of the smallest patches we could measure was on the order of milliseconds, the same time scale as responses by chemosensory receptor cells. Patch length, patch frequency and peak-to-trough fluctuations in patch concentration all may provide information on the distance or direction a detecting organism is positioned away from the source of chemical emission. Whether, or not, an organism tunes to specific properties of patches and extracts this information depends on: (1) the complexity of the natural habitat in generating turbulent eddies and vortices that entrain stimulatory molecules, and (2) the life history traits of the responding organism.

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Feature Extraction for Chemical Gradient Search. Jelle Atema, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

Temporal sampling of local odor features by receptor cells and organs may allow the animal to reconstruct a spatial gradient from which source distance and direction can be estimated.
Chemical signals disperse in the environment via molecular diffusion through the medium and by flow of the medium. The medium may be air or water. Flow tends to be viscous at spatial scales below 1 cm and turbulent at larger scales depending on flow velocities. Small animals and the noses of large animals operate in viscous environments. Large-scale dispersal results in turbulent odor plumes characterized by patchy distributions of areas with higher and lower odor concentration. The chaotic nature of turbulence requires that spatial or temporal averages of stimulus parameters be taken before behaviorally useful spatial gradients can be established. The choice of parameters probably depends on the animal's behavioral capabilities and purpose. Which stimulus parameters are behaviorally most significant can only be studied when odor distributions are measured at the proper spatiotemporal scale.
Physical structures and flow-controlling behavior of chemoreceptor organs result in great differences in boundary layers and diffusion access paths for odor molecules. Organ morphology can be seen as an adaptation to utilize boundary/diffusion layers as a first (temporal) filter to sample chemical signals in the environment. Physiological properties of receptor cells determine their sensory function as spectral (odor quality) and temporal (odor intensity) filters. The great diversity of filter properties seen across a population of receptor cells include tuning spectrum, response function, self adaptation and disadaptation, cross adaptation and mixture suppression. From this neural diversity the CNS extracts behaviorally useful information. The process of feature extraction remains unknown. Odor signal features include mixture composition and rates of intensity change. Responses of ensembles of receptor cells to controlled stimulus features of odor plumes are needed to understand what constitutes a chemical signal. Recently developed techniques can be applied to help solve problems of stimulus measurement control and neural feature extraction.

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Temporal and Spatial Distribution of a Pheromone Signal: Cues for Orientation of Flying Moths. RING T. CARDÉ, MARK A. WILLIS, JOSEPH S. ELKINTON (Univ. Massachusetts) and JOHN MURLIS (Ministry of the Environment, U.K.).

Moths navigate a course to a source of female-emitted pheromone mainly by flying upwind and they determine wind direction by watching the ground pattern and compensating for wind-induced drift. The distribution of pheromone in wind, however, rarely would allow a moth to follow a direct path to a source of pheromone, because the direction upwind and the direction along the plume's long axis are aligned only infrequently. Because of this constraint, gypsy moths do not routinely or quickly locate pheromone sources 20 meters or more distant. The temporal and spatial structure of pheromone plumes has been investigated by simulating plumes with ionized air. When ions and pheromone were released a few cm apart, the downwind appearance of bursts of ions was well correlated with detection of pheromone with an electroantennogram, validating the ion signal as a good simulation of pheromone intensity. Ion plumes in forests and open grassy fields are quite different, in part due to the lowered wind speed and turbulence in the forest. A flying male encounters an highly intermittent signal, and there is some evidence suggesting that such fluctuations (burst return periods < 1 sec.) are important to a male's orientation response.

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Steroidal and Prostaglandin Sex Pheromones Selectively Stimulate the Medial Olfactory Tracts of Male Goldfish. PETER W. SORENSEN (Dept. of Fisheries & Wildlife, University of Minnesota, St. Paul, MN 55108), TOSHIAKI J. HARA (Freshwater Institute, Winnipeg, Manitoba R3T 2N6, Canada), NORMAN E. STACEY (Dept. of Zoology, University of Alberta, Edmonton, Alberta T6G 2E9, Canada).

Goldfish possess paired pedunculate olfactory bulbs which are connected to the telencephalon by relatively long olfactory tracts each of which is comprised of two major subtracts: the medial olfactory tract and the lateral olfactory tract. Histological, behavioral, and endocrinological studies have suggested that the medial, and not the lateral olfactory tracts, convey information relating to sexual behavior and pheromones. In this study we examined the functions of these tracts by recording from them while exposing mature goldfish to a variety of olfactory stimulants including: steroidal and prostaglandin sex pheromones, a bile salt, and L-serine (an amino acid which functions as a feeding attractant). Only the medial tracts responded to the sex pheromones while both tracts responded to L-serine, bile salt, and mechanical disturbances associated with odor application. These findings confirm that peripheral responses to water-borne steroids and prostaglandins are conveyed centrally by the medial olfactory tracts and suggest that the medial and lateral portions of the fish olfactory system have separate, specialized functions.

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Neuroendocrine Mediation of a Pheromone Evoked Sexual Behavior in the Blue Crab (Callinectes sapidus) DEBBIE WOOD, CHARLES D. DERBY* (Georgia State University, BARBARA BELTZ (Wellesley College)**, RICHARD A. GLEESON (Whitney Marine Laboratory)

The male blue crab produces a sterotyped courtship display in response to a pheromone produced by the female. This behavior consists of both postural and rhythmic components. This system is an excellent model for the study of chemosensory and motor integration because it is a simple rhythmic behavior elicited by a single chemical. This model has also yielded interesting results related to the neurohormonal modulation of the behavior. In vivo electromyogram studies have revealed parameters of the motor program of the rhythmic behavior which allow for in vitro eletrophysiological discrimination of the pattern of motor activity. Bioassays of neuromodulators have shown that the monoamine dopamine and the neuropeptide proctolin are capable of producing the postural and rhythmic behavioral components respectively. We are currently undertaking electrophysiological studies to detect changes in motor neuronal output in the presence of proctolin and dopamine. Immunocytochemical studies are also in progress to determine the location of proctolin-like and dopamine-like immunoreactivity. These experiments will allow us to identify potential neurosecretory cells in this system.

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Influence of the D-2 Dopamine Receptor Agonist Quinpirole on the Odor Detection Performance of Rats Before and After Spiperone Administration. RICHARD L. DOTY and JUDITH M. RISSER (Smell and Taste Center, Hospital of the University of Pennsylvania, and Department of Otorhinolarygnology and Human Communication, School of Medicine, University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA)

The influence of five doses of the D-2 receptor agonist quinpirole (.025, .05, .075, .10, and 0.20 mg/kg IP) on the odor detection performance of 21 adult male Long Evans rats was assessed using high precision olfactometry and a go/no-go operant task. Additionally, 10 rats were pre-treated with the D-2 receptor antagonist spiperone (0.62 mg/kg IP) and their performance monitored following quinpirole administration. Treatments were administered every 3rd day in a counterbalanced order, with the quinpirole injections occurring 15 min before, and the spiperone injections 35 minutes before, the 260-trial test sessions. Quinpirole injection resulted in a dose-dependent decrease in odor detection performance, as measured by the percentage of correct trials and by the non-parametric signal detection sensitivity index SI. Prior treatment with spiperone eliminated these effects. Dose-related influences of quinpirole on (a) the average latency to initiate a detection response (i.e., the S+ response latency), (b) the total session duration, and (c) the number of aborted trials were also eliminated or greatly attenuated by prior spiperone injection. These results suggest that D-2 receptors may be involved in the modulation of odor detection performance and related behaviors.

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Changes in Neural Metabolic Activity after Unilateral Odor Preference Conditioning in 6-day-old Rats
LAUREN FRAZIER-CIERPIAL (Duke University),
WARREN G. HALL (Duke University)

In 6-day-old rat pups, olfactory information is processed and stored unilaterally (Kucharski & Hall, Science, 238:786-8,1987) which effectively isolates olfactory learning at this age. This isolation was utilized to make within brain comparisons of neural changes in the unilateral acquisition of an odor preference. During training, 6-day-old pups were exposed to repeated pairings of cedar odor and milk delivery. One nostril was plugged to unilaterally restrict odor exposure. Pups in the experimental and control conditions were tested with the trained or untrained naris open, respectively. The resulting changes in neural metabolic activity were mapped in the olfactory bulb, olfactory cortex and related structures using semiquantitative ^{14}C 2-deoxyglucose (2-DG) autoradiography. Topography at given section levels was closely matched using templates drawn from corresponding Nissl-stained sections and then averaged for both conditions. Comparisons of average images revealed subtle differences due to acquisition of the odor preference. The learned odor preference resulted in areas of greater uptake in discrete locations within the olfactory bulb and anterior piriform cortex. In addition, particularly dramatic differences were found in mid-levels of the anterior olfactory nucleus. Here, learning resulted in greater uptake over relatively wide locations which was maintained over a large portion of the structure. Such findings suggest restricted changes in metabolic activity associated with learning in many structures involved in olfactory information processing.

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Learned Food Aversions in Radiotherapy Patients. RICHARD D. MATTES (Monell Chemical Senses Center), WILLIAM POWLIS (Hospital of the University of Pennsylvania), CATHY ARNOLD and MARCIA BORAAS (Fox Chase Cancer Center).
The prevalence and nature of learned food aversions (LFA) have been documented in cancer patients receiving chemotherapy. Animal studies indicate that radiation treatments may be particularly problematic with respect to LFA formation, but little is known about this problem in radiotherapy patients. Issues of a) patient risk status, b) the incidence of LFA, c) food targets, d) the critical period for LFA formation, e) LFA persistence and f) clinical consequences of LFA are currently under study in patients administered radiation treatments to either the head and neck or abdominal regions. Patients are assessed prior to the initiation of therapy, every other day for the first week, weekly for the next five weeks and at months 2, 4 and 6. LFA are documented via open-ended questionnaires, acceptability ratings for foods ingested in the 48-hour period surrounding the first treatment day and a food intake procedure. Preliminary observations reveal an incidence of newly formed (i.e., post-treatment initiation) LFA of approximately 50%. LFA are more common in patients treated in the abdominal region compared to the head and neck and patients with a pre-treatment history of LFA are approximately twice as likely to form new LFA as those with a negative prior history. Items representing all common food groups have been targeted, however certain items are particularly problematic (e.g., sweets, meats, caffeinated beverages). Foods ingested as much as 24-hours before or after the first treatment can be targeted, but the duration of aversions is generally short (1-4 months). Patients with LFA tend to report a loss of appetite and body weight more often than those not forming LFA, but no effect of this treatment sequela on progression of disease has been noted. Thus, as is the case with chemotherapy patients, LFA pose a greater threat to the quality of life than clinical status.

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Sour taste perception with simple food systems
JAYA CHAUHAN (Oxford Polytechnic)
Perceived taste intensity and intensity relative-to-ideal of citric acid in an apple drink were assessed subsequent to 1) rinsing with deionised water 2) consumption of plain mashed potato prior to rinsing 3) consumption of mashed potato seasoned with white pepper prior to rinsing. Eighteen male subjects, students and staff members, ranging in age 18 to 35 years (mean age, 27.2 yrs) participated. All assessments were made in duplicate in six separate sessions. Using 100-mm graphic scales, subjects rated perceived intensity and intensity relative to ideal. Subjects showed a decreased sensitivity and increased preference for acid in drink following consumption of plain potato prior to rinsing compared to rinsing only and consumption of seasoned potato prior to rinsing. It would thus appear that the pepper in the food system heightened sour intensity perception to negate the effect of plain potato. White pepper, unlike black pepper produces very little taste sensation, its major quality being irritancy illicited largely by piperine. Whether the present observation is confined to piperine as the oral chemical irritant and sour taste quality merit investigation. Subjects in the present study were not homogeneous in their consumption of hot food and it is likely that consumers and non-consumers of hot food may yet give different results. Certainly, this study demonstrates the importance of using ecologically valid study designs for extrapolation of data to real eating situations. Consumption of a simple food such as potato markedly suppressed sour intensity perception and is a factor which needs consideration in the formulation of food products where sourness is an important hedonic quality.

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Study of Gustation and Olfaction in Culinary Experts. ALAN R. HIRSCH (Smell and Taste Treatment and Research Foundation), MICHAEL JUTOVSKY. [1]

To study olfactory and gustatory ability in generally recognized culinary experts, ten male nondiabetic chefs underwent testing which included the UPSIT, the Unilateral Connecticut Home Olfactory Test, isoamyl acetate adaptation testing, smell suprathreshold detection testing, smell suprathreshold recognition testing, smell suprathreshold identification testing, isoamyl acetate intensity testing, unilateral carbinol threshold testing, unilateral pyridine threshold testing, taste suprathreshold identification testing, taste threshold testing, and electrical unilateral gustometry testing. Chefs segregated into two groups; six who had excellent olfaction and taste and could not adapt to continuous olfactory stimuli, three who had poor olfactory ability and readily adapted to odors, and one fell into a borderline zone. There was correlation in UPSIT and age correlated UPSIT and ability to taste urea. Adaptation testing was directly correlated to olfaction.

[1] Thanks for assistance in this paper to J. Amoore, M. Kare, L. Barotshuk, D. Deems, W. Cain, and R. Szczesiul.

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Study of Gustation and Olfaction in Culinary Experts— Study of Taste. ALAN R. HIRSCH (Smell and Taste Treatment and Research Foundation) MICHAEL JUTOVSKY. [1]

To study gustatory ability in generally recognized culinary experts, 10 male nondiabetic chefs underwent various testing of olfaction and gustation. Other than grossly, there was no correlation between ability to taste and ability to smell. Seventy percent of chefs had a reduction in ability to taste. Twenty percent of chefs had difficulty detecting salt. Seventy percent had significant difficulty differentiating sour from bitter.

[1] Thanks for assistance in this paper to J. Amoore, M. Kare, L. Bartoshuk, D. Deems, W. Cain, and R. Szczesiul.

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In-vivo voltage-clamp effects on rat tongue transepithelial current and neural response during salt stimulation.. GERARD HECK, KRISHNA PERSAUD, and JOHN DESIMONE (Medical College of Virginia, Virginia Commonwealth University, Dept. of Physiology, Richmond, VA 23298, U.S.A.)

An adaptation of epithelial techniques to the rat allows the simultaneous measurement in vivo of transepithelial electrical characteristics and neural responses. A vacuum-attached stimulation chamber isolates a patch of tongue. Voltage across the epithelial patch is measured between an electrode in the chamber and another embedded in the tongue muscle. Auxillary electrodes pass current through this patch. This voltage-clamped preparation is similar to but not identical with preparations used to demonstrate electric taste. A variable current here is confined to a well-defined epithelial patch and maintains a constant voltage across it. When the epithelium is clamped at 0 volts, both fast and slow inwardly-directed components are seen in the current response. The neural response also shows two components: a fast component building to a peak value and a second slow component declining to an adapted state. Both the neural and current responses are similarly concentration dependent. The amplitude of the peak neural response correlates well with the amplitude of the fast current at the same time point. The neural response adapts with a time constant nearly identical to that of the slow component of the transepithelial current. We propose that the fast current represents flow through apical ion channels leading to cell depolarization and that the slow current is a repolarization current involved in adaptation. Clamping the epithelium at negative potentials increases the neural response; clamping at positive levels reduces but does not abolish the neural response. This is similar to electric taste and consistent with an ion-channel transduction model.

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The NaCl taste response: a model to integrate ion transport mechanisms. SHEELLA MIERSON and MARK L. FIDELMAN (Medical College of Virginia, Virginia Commonwealth University, Dept. of Physiology, Richmond, VA 23298, U.S.A.)

The tongue epithelium including taste buds is a complex structure with multiple pathways for ion transport. Transport of an individual ion through one type of channel is challenging enough to describe mathematically. With several ions and barriers, the mathematics is more complicated; intuition is unreliable. To provide a framework for integrating multiple mechanisms and complicated topology, we used a mathematical model of a transporting epithelium. The model has 4 membranes (apical, basolateral, tight junction, and basement) and 3 transported ions (Na^+, K^+, and Cl^-). It calculates ion flows and related electrical events in the rat tongue. The model successfully simulates steady-state transepithelial electrical measurements observed in vitro for a wide range (50-2000 mM) of mucosal NaCl concentrations. In response to a prolonged hyperosmotic NaCl stimulus, the model predicts intracellular depolarization followed by repolarization. Since the intracellular potential has been identified with the taste receptor potential, the depolarization and subsequent repolarization may explain both the phasic and tonic components of taste nerve excitation in response to a salt stimulus. This sequence of depolarization and repolarization is due to the organization of the component membranes and cannot be predicted from properties of the individual membranes in isolation. The results indicate that the specific topology of the epithelium in which the taste buds are embedded is important in understanding taste transduction. The analysis was begun at the simplest level, with one cell type and 3 diffusible ions, but can be extended to incorporate various transport mechanisms and cell types. This modeling approach is flexible enough to easily test different specific models. We anticipate that the method will be useful to examine interactions between different tastants.

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Peripheral taste responses and amiloride sensitivity in genetically hypertensive rats. BRADLEY K. FORMAKER & DAVID L. HILL (University of Virginia)

In two-bottle preference/aversion tests, the spontaneously hypertensive rat (SHR) tolerates higher concentrations of NaCl than the normotensive Wistar-Kyoto rat (WKY). In contrast, the inbred Dahl salt-sensitive (S/JR) and salt-resistant (R/JR) rat show similar NaCl preference/aversion functions. Although others have studied the electrophysiological consequences of these behaviors, we wanted to investigate whether differences in preference/aversion functions could be attributed to differences in amiloride sensitivity. Multifiber electrophysiological taste responses were recorded from the chorda tympani nerve in the SHR, WKY, S/JR and R/JR rat. Responses to a concentration series (0.05M to 0.5M) of NaCl, NaAcetate (NaAc), KCl, NH_4Cl and $CaCl_2$ were recorded before and after the lingual application of amiloride hydrochloride. Relative responses to NaCl were equivalent between SHR and WKY rats, before and after amiloride. Furthermore, relative responses to NaAc, NH_4Cl, KCl and $CaCl_2$ did not differ between SHR and WKY rats. Thus, no difference in chorda tympani activity was evident between SHR and WKY rats. By comparison, relative response magnitudes to NaCl were greater in R/JR than S/JR rats. However, the magnitude of amiloride suppression was equivalent between these two strains. KCl responses were equivalent before amiloride application; however, post-amiloride KCl responses were greater in R/JR than S/JR rats. Responses to NaAc, NH_4Cl and $CaCl_2$ were equivalent between R/JR and S/JR rats. These results suggest that NaCl preference/aversion functions in genetically hypertensive rats are not related to amiloride sensitivity. Furthermore, NaCl intensity coding appears to be mediated by CNS responses, rather than chorda tympani responses, in the S/JR, R/JR, SHR and WKY rat.

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Detection Thresholds of Sodium Salts is Related to Molecular Conductivity of the Anion. SUSAN S. SCHIFFMAN, ALVIN L. CRUMBLISS, ZOE S. WARWICK (Departments of Psychiatry and Chemistry, Duke University)

Recent studies indicate that salt taste transduction is mediated in part by transport of sodium ions through apical sodium channels. However, an overview of these studies reveals that amiloride inhibits only part of NaCl responses. This suggests that pathways or receptors that are insensitive to amiloride are also involved in salt taste transduction. The relative degree of inhibition by amiloride is greater when the salt concentrations are hyperosmotic than when they are hyposmotic. Thus amiloride insensitive pathways play a greater role at hyposmotic salt concentrations. The purpose of this study was to gain further insight into the factors that account for hyposmotic salt taste transduction. Detection thresholds and recognition thresholds for salty taste were determined for ten sodium salts. Sodium citrate had the lowest detection threshold (0.0005M). The highest threshold was for sodium ascorbate which at 0.0041M was 8.2 times greater than the threshold for sodium citrate. Detection threshold concentrations for sodium salts were found to be linearly correlated (r=.91) with the molar conductivity of the anion of the salt. Molar conductivity (expressed in ohm^{-1} cm^2 $mole^{-1}$) were obtained from data tabulations at infinite dilution and 25°C. Molar conductivity is directly proportional to both ionic mobility and ionic charge, i.e. the electrical charge carried by the anion per unit time. This suggests that an anion pathway is a component of the amiloride-insensitive pathway for sodium salts.

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Axon addition and growth in the recurrent gustatory nerve of the channel catfish. SUSAN GREENER, MARY WOMBLE & THOMAS E. FINGER (Univ. Colorado School of Medicine & Rocky Mountain Taste & Smell Center, Denver, CO 80262).

The recurrent branch of the facial nerve is a pure gustatory nerve, carrying facial nerve fibers which innervate the taste buds on the animal's flank. This nerve contains no admixture of spinal or trigeminal components. Since the receptor space innervated by this nerve expands considerably as the a fish grows, we wanted to determine whether the fiber number and spectrum of this nerve changes as a function of growth. Toward this end, a segment of the recurrent nerve was removed at its point of exit from the skull in fishes of different sizes, from 5.1 cm to 18.5 cm standard length. The tissue was processed for electron microscopy and a random photograph taken at 6600X magnification from every 115 μm grid square. In order to measure the diameter of unmyelinated fibers, another series of photographs was taken of selected areas at a magnification of 11,500X. The total number of myelinated and unmyelinated axons and diameter of myelinated axons was estimated from the random photographs. Both the total number of axons and mean axon diameter increase as a function of fish size. This is true of both the myelinated and unmyelinated populations although the ratio of myelinated to unmyelinated fibers remains roughly constant. Selected data are given in the table following:

Size of Fish	Total # Axons	Mean Axon Diameter	
		Myelinated	Unmyelinated
5.1 cm	10,000	0.5 μm	0.22 μm
8.2 cm	19,200	0.8 μm	0.27 μm
18.5 cm	61,200	1.1 μm	0.33 μm

These data indicate that the peripheral nerves continue to grow throughout the animal's life, both increasing the mean diameter of axons and by adding new axons to the gustatory nerves.

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Autoradiographic demonstration of neurons migrating from the olfactory neuroepithelium. P.P.C. GRAZIADEI and A.G. MONTI GRAZIADEI (Biological Science, Florida State University, Tallahasse, Florida).

It is well known that the olfactory neuroepithelium provides neurons for the olfactory organ proper, the vomeronasal organ and the nervus terminalis system. Previous studies of partial and total bulb removal in adult rodents have shown that cells can migrate out from the neuroepithelium and along the olfactory nerve toward the olfactory bulb. The neuronal nature of these migrating cells was documented with the electron microscope (Monti Graziadei and Graziadei, 1983). In the present investigation, adult unoperated mice received one injection of 2.5 uC/gbw of 3H-thymidine (specific activity 6.7 Ci/mmol). At survival times ranging from 24 hours to three days, the mice were sacrificed by perfusion. The heads were embedded in paraffin, serially sectioned and the sections were processed for autoradiography according to standard procedures. Autoradiographically labeled cells were present in the basal layer of the neuroepithelium and in the lamina propria of the olfactory mucosa. The autoradiographically labeled cells, identified as neurons, observed in the lamina propria were either collected in small clusters or isolated along the fila olfactoria. The results of these study suggest that the olfactory neurogenetic matrix of adult mice can produce neurons the destiny of which lies outside the neuroepithelium itself. The final station of these neurons is at present under investigation.

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Projections of Olfactory Receptor Neurons in Rainbow Trout.

DAVID R. RIDDLE AND BRUCE OAKLEY (Department of Biology and Neuroscience Program, Neuroscience Lab Bldg., University of Michigan, Ann Arbor, MI 48109.)

In many sensory systems the pattern of projection of the primary receptor neurons to their targets in the central nervous system is important for coding sensory information. The organization of olfactory receptor neurons and their projections to the olfactory bulb is not yet clearly understood, and its importance for coding olfactory quality remains to be established. In the rainbow trout, *Salmo gairdneri*, we have studied the organization of the primary olfactory neurons in the olfactory mucosa, and the pattern of their projections to the olfactory bulb. We have used HRP to label the receptors on 2-3 of the 10-14 lamellae of the olfactory rosette in adult fish. Labeled axons were grouped in the olfactory nerve, but diverged as they entered the olfactory bulb and spread broadly over large areas of the glomerular layer. The patterns of labeled projections in the glomerular layer were different in different individuals, even when the same region of the olfactory rosette was labeled in both fish. Thus it appears trout lack a standardized topographic projection like that proposed for amphibians and mammals. When both rosettes were labeled in a single fish, the pattern of labeled projections appeared quite similar, even when HRP was placed on different regions within the two rosettes. This was not due to bilateral projections, since HRP placed on one rosette labelled projections only in the ipsilateral olfactory bulb. There appears, then, to be significant redundancy in the olfactory system in trout, such that receptors located throughout the mucosa project to the same regions in the glomerular layer of the olfactory bulb. We are now attempting to label receptors in small regions within a single lamella to determine if some subtle topography exists which was obscured when entire lamellae were labelled.

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The Direct Determination of Trace Metals in Olfactory Bulbs by Inductively Coupled Argon Plasma-Mass Spectrometry.

J. EVANS, L. HASTINGS (Dept. of Environmental Health, Univ. of Cincinnati), T. E. DAVIDSON and J. A. CARUSO (Dept. of Chemistry, Univ. of Cincinnati).

The olfactory system has been shown to undergo pathological as well as functional changes during the early stages of degenerative diseases such as Alzheimer's and Parkinson's Disease. It has also been demonstrated that the olfactory system may provide a route of entry for toxic compounds into the central nervous system. To study a possible relationship between these two observations, the examination of the metal content of olfactory bulbs from deceased human subjects who had been diagnosed as having a degenerative brain disease is proposed. To permit multi-elemental analysis, inductively coupled argon plasma interfaced to a quadruple mass spectrometer was used. Preliminary to the use of human tissue, rat olfactory bulbs were used to work out digestion procedures and to determine the sensitivity of the method. In this preliminary study three Long-Evans rats received a unilateral instillation of 15 ul of 1 mM $CdCl_2$ into the right naris and three received 15 ul of 1 mM $AlCl_3$. One week after exposure, rats were sacrificed and the olfactory bulbs removed. Bulbs were weighed, digested in 2 ml HNO_3 and diluted to 25 ml. Measurement of Al was not possible due to interference encountered in its mass range. However, Cd concentrations were 20 to 60 times higher in exposed vs. control olfactory bulbs. Background concentrations of Ni, Cu, Zn, and Rb were comparable across all samples. These results demonstrate that the measurement of a variety of metals in micro samples of brain tissue are feasible. This method will now be applied to human olfactory bulbs to examine the possibility that abnormally excessive or reduced metal content correlate with a particular degenerative disease.

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Pathological changes in the Olfactory Epithelium of Patients with Alzheimer's Disease. B.R. TALAMO, R.A. RUDEL, K. KOSIK*, V.M.-Y LEE*, S. R. NEFF, L. ADELMAN and J.S. KAUER (Tufts Med. Sch., N.E.M.C., Harvard Med. Sch.*, Boston, MA. and Univ. of Penn., Phila. PA).

Patients with Alzheimer's disease (AD) are reported to show olfactory deficits (Doty et al., Br. Res. Bull. 1:597, 1987). Olfactory epithelia obtained at autopsy from 9 confirmed AD patients and 14 aged non-AD patients were studied with immunohistochemical methods using antibodies (Ab's) directed against olfactory marker protein (F. Margolis) to identify sensory areas, and Ab's against various cytoskeletal proteins, particularly those which stain neurofibrillary tangles in AD brain. Receptor cells did not stain with Ab's to intermediate filaments, including neurofilaments, cytokeratin, GFAP, and vimentin. Axon bundles stained only with Ab to the non-phosphorylated form of the mid-sized neurofilament subunit. No neurofibrillary tangles or plaques were seen in the epithelium of AD patients using conventional staining techniques or Ab's which revealed tangles in cortex and the anterior olfactory nucleus. However, olfactory epithelium from AD patients showed two striking alterations: (1) a massive proliferation of neurites below the basal lamina and projecting into the olfactory epithelium, and (2) differential staining of the abnormally placed neurites and of some olfactory nerve bundles. These neurite accumulations were intensely stained by Ab's specific for each of the three subunits of neurofilament protein, particularly the heavily phosphorylated forms, and with the Ab ALZ-50 and Ab to tau, a microtubule-associated protein which is the major antigenic epitope in neurofibrillary tangles and dystrophic neurites in AD. The proliferation of neurites and the antigenic profile of the structures in olfactory epithelium has some features in common with dystrophic neurites of AD, and provides evidence for abnormal expression of both tau and neurofilament protein in AD. Olfactory epithelium is a source of affected tissue which may be obtained through biopsy for further studies of the mechanism of AD and for the possible development of diagnostic tests.

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Complete Recovery of Deficit in Total Olfactory Neuronal Number by Restoration of Thyroid Function in Post-weaning Rats MARK PATERNOSTRO AND ESMAIL MEISAMI (Dept. of Physiol. & Biophys., Univ. of Illinois, Urbana, IL 61801)

In the 1988 AChemS meeting, we showed that in hypothyroid suckling rats (days 1 to 25), the marked increase in area of olfactory epithelium and total number of olfactory receptor neurons that normally occurs during this period is severely retarded (40%, $p<0.01$)). Our more recent results have expanded this picture: if thyroid deficiency is continued beyond weaning, the normal increase in olfactory neuron number ceases entirely accompanied with thinning of the olfactory epithelium. However, if after weaning thyroid functions is permitted to resume, by replacing the water containing propylthiouracil (PTU) with tap water, olfactory epithelial growth resumes both in terms of area and total neuronal number, resulting in complete recovery of these parameters within two months. This growth plasticity of the olfactory epithelium and its neurons is in marked contrast to the effects in CNS. We have obtained these results by using morphometric techniques and direct counts of the nuclei of olfactory neurons in Hematoxyline-Eosin stained serial sections. However we need to know the percentage of mature vs. immature olfactory neurons in the olfactory epithelium of the hypothyroid and rehabilitated animals. In this presentation, in addition to the above-mentioned morphometric and cell counting results, we present further quantitative data from ongoing protargol staining and immunocytochemical (olfactory marker protein, OMP) studies in normal, hypothyroid and rehabilitated rats. The protargol stained sections permit quantification of the olfactory knobs which are associated with mature neurons. These estimates will be compared to those obtained from OMP stained sections which selectively differentiates the mature olfactory neurons from the immature population.

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Nasal Airflow Patterns. LEOPOLD, D.A., D.E. HORNUNG, R.L. RICHARDSON, S.L. YOUNGENTOB, M.M. MOZELL and P.W. SCHERER (SUNY-HSC, Syracuse, N.Y.)

A changing density detection technique was used to quantify the air flowrate through an anatomically correct model of the human nasal passageways. So that only a small region of the model was viewed at any one time, an I125 gamma ray point source was placed one side of the model and a narrow beam collimated detector was placed on the other. To determine nasal airflow, the model was first filled with xenon gas. Room air was then drawn at a controlled flow rate through the external naris and into the model. As the xenon was removed from the model, the absorption of the gamma rays was reduced, and the number of gamma rays received by the detector increased. The rate at which the count rate increased was a measure of the flow through the particular portion of the model being studied. A modification of Beer's Law was used to calculate the actual flow rate from the rate at which the counts increased. To determine the recording locations, a perpendicular line was drawn from the anterior cribriform region across the anterior middle turbinate down to the palate. The detector was moved in 2.7 mm increments along this line and the flow rate determined at each position. When the xenon gas was removed from the model at rates of 15, 6, and 2 l/min, the air flowrates in the superior regions of the nasal cavity were somewhat slower than the flows seen in the other nasal regions. This observation likely reflects the relationship between cross-sectional area and flowrate since the superior regions (the area leading to the olfactory receptors) are somewhat narrower than the rest of the nasal cavity. Also, contrary to previous predictions, this pattern of airflow was not dramatically affected by changing the flowrate through the entire model.

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Mixture Interactions in Olfaction: Behavioral Responses to Binary Mixtures and Single Compounds in the Spiny Lobster. PETER C. DANIEL, CHARLES D. DERBY (Georgia State University)*

Do mixture interactions occur in olfaction? We are examining this question at the behavioral and physiological levels using as a model the spiny lobster. In behavior, the subject of this poster, it is necessary to establish a reliable measure of response magnitude before determining whether responses to a mixture can be predicted from responses to each of the components of the mixture. This has been accomplished using the antennule flick response: the rate of flicking increases with the introduction of various chemicals (AMP, betaine, cysteine, succinate, taurine) commonly found in lobster food. Chemical stimuli are presented using a head set apparatus (see Daniel and Derby, 1988, Chem. Senses13:385-395), which reduces the likelihood of higher flick rates evoked by nonchemical stimuli characteristic of other stimulation methods. Flick rate does not increase progressively with the concentration of the introduced chemical stimulus. Rather, the probability of an above baseline flick rate increases with dose. The relationship is best modeled by a probit curve and not by linear or kinetics equations and is characterized by a shallow slope. Responses to binary mixtures of the above five chemicals over a wide range of concentrations are currently being tested. Results of these experiments will be compared to predicted responses based on dose-response curves for chemicals presented singly in order to measure whether significant interactions are occurring.

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Host Volatiles as Gustatory Stimuli for Phytophagous Insects. B.K. MITCHELL, B.G. MCCASHIN (Department of Entomology, University of Alberta), P.J. ALBERT (Department of Biology, Concordia University).

Recent work on galeal gustatory sensilla of the Colorado potato beetle (Leptinotarsa decemlineata) shows that a single cell produces the primary response to freshly ground potato leaf sap (Mitchell, et al., 1989). Attempts to characterize the compound(s) responsible for the strong stimulation led to testing of a vacuum distillate of potato leaf sap on these same sensilla. The distillate proved to be a very effective stimulus of the primary cell, and the recordings were even cleaner (more unicellular) than those with fresh sap. Since the distillate contains none of the compounds normally considered as effective stimuli for insect taste (eg. sugars, amino acids, alkaloids) this finding may represent the discovery of an entirely new group of potential stimuli for taste receptors in insects.

The generality of this phenomenon has been investigated by testing distillates, residues and fresh host extracts on selected gustatory sensilla in larvae and/or adults of a number of phytophagous insects. Results demonstrate that while sensitivity to host volatiles is not a universal capability of insect taste receptors, the phenomenon occurs often enough that it should be seriously considered in investigations of host recognition and feeding behavior.

REFERENCE

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A New Trichoplusia ni Antennal Receptor Cell that Responds to Attomolar Concentrations of a Minor Pheromone Component

M. S. MAYER AND R. W. MANKIN

Insect Attractants, Behavior, and Basic Biology Research Laboratory, USDA, ARS, Gainesville, Florida, 32604, USA

An investigation of quantitative sensitivity and discrimination of individual Trichoplusia ni olfactory receptor neurons in relation to sexual behavior has yielded findings of special interest. Previously, two olfactory receptor neurons within separate sensilla have been demonstrated to respond with great sensitivity either to (Z)-7-dodecen-1-ol acetate or (Z)-7-tetradecen-1-ol acetate, two of the six components emitted by the female. Another receptor neuron within a third type of sensillum now has been characterized that responds to a third component emitted by the female, (Z)-9-tetradecen-1-ol acetate. Its response threshold of 2×10^{-18} M is lower than the response threshold for the receptor neurons for (Z)-7-dodecen-1-ol acetate, the major pheromone component, and (Z)-7-tetradecen-1-ol acetate, which are near 1×10^{-15} M. As with the other receptor neurons, it also responds to high concentrations of the other components. This suggests that (Z)-9-tetradecen-1-ol acetate has particular significance to the behavior of the male.

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Pheromonal Regulation of Molting in Lobsters, Homarus americanus. DIANE F. COWAN (Boston University Marine Program)

Sensitivity to exogenous cues such as temperature, photoperiodicity and food availability influence the hormonal regulation of molting in lobsters, Homarus americanus. Synchronous molting is expected based on environmental cues. However, when two mature male and five female lobsters were observed in a 4000 gallon aquarium, the females delayed and staggered the timing of their molts at intervals throughout the summer to mate with the dominant male. The experiment was repeated three times and in each case female molt staggering led to a mating system of serial monogamy. Molt staggering implies behavioral control over the female molt cycle, perhaps via pheromones. I suggest that the dominant male regulates the timing of female molts via primer pheromones, thereby increasing his seasonal mating success. If male lobster pheromones influence the timing of mature female molting and therefore mating, this will be the first evidence for the social control of reproductive receptivity in any invertebrate.

Partial financial support for this work was provided by the BU Marine Program, the Whitehall Foundation and NSF (BNS8413661) to Jelle Atema.

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Mutations Restricted to Genes in the MHC Alter Body Odor. KUNIO YAMAZAKI, GARY K. BEAUCHAMP (Monell Chemical Senses Center, Phila., PA); JUDITH BARD and EDWARD A. BOYSE (Memorial Sloan-Kettering Cancer Center, New York, NY).

The Major Histocompatibility Complex of genes (MHC) in the mouse contains about fifty closely linked genes, many of which control graft rejection and other immune responses. Previous studies have demonstrated that these genes are involved in provisioning an animal with an olfactory signal characteristic of the animal's genotype. In order to evaluate whether very small differences in the genetic make-up of mouse strains are responses for individual variation in chemosensory identity, we have studied the ability of trained mice to distinguish the odor of non-mutant strains from that of congenic mutant strains differing only by mutations of two types of MHC genes, those of Class I or Class II. Olfactory distinctions between all mutants and non-mutants were observed in the Y-maze. Surprisingly, there was no evidence of discrimination among many of the mutants even though these mutants differ at one or several DNA base pairs.

The extent to which the recognition of such MHC-determined odor differences could operate across species barriers was next examined. Using an automated olfactometer and mouse odor, it was found that rats can make the same MHC discriminations (e.g., between mutants differing at a single MHC gene), of which mice are capable. No known group of linked genes exhibits the same diversity as the MHC.

The current studies demonstrate that variation restricted to a few DNA base pairs of a single gene is reflected in variation in body odor. Each mouse in nature may have a unique odortype specified by its unique constellation of MHC genes.

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Chemosensory-guided Behavior and Anatomy of the Nasal Cavity in the Axolotl. HEATHER L. EISTHEN*, DALE R. SENGELAUB*†, and JEFFREY R. ALBERTS* (*Department of Psychology and †Program in Neural Science, Indiana University).

Feeding and social/sexual behavior in the axolotl (Ambystoma mexicanum), a neotenic salamander, are chemically-mediated. During food-searching, axolotls adopt a distinct posture in which the head is tilted nearly perpendicular to the substrate, thereby better exposing their dorsally-located nostrils. The frequency of axolotls' head-tilting, but not locomotion, increased significantly during 4-hr observational tests following 5- or 10-day food deprivation or addition of a suspension of ground food pellets to the aquarium. In separate experiments, adult males' locomotor activity was measured during exposure to clean water, or water from the bowls of gravid females, nongravid females, or adult males. Activity decreased in the presence of gravid female water, relative to clean-water controls, but was unaffected by nongravid female or male water. We are also beginning anatomical investigations of the axolotl olfactory system. Three-dimensional reconstructions of the head reveal an elongate nasal cavity with a lateral pouch. In histological sections we observe apparently separate receptor populations along the medial wall of the nasal cavity and in the lateral pouch, corresponding to the olfactory and vomeronasal receptor loci in other salamanders. We are currently conducting HRP tract-tracing to determine the connectivity and projection distribution of these receptor populations.

Supported by NIMH grant MH-28355.

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Chemical Defenses of Azalea Lace Bugs Against Two Insectivorous Birds. J. RUSSELL MASON (Monell Chemical Senses Center and Denver Wildlife Research Center, Philadelphia, PA), WILLIAM R. LUSBY (Insect and Nematode Hormone Laboratory, Agricultural Research Service [ARS], Beltsville, MD), JAMES E. OLIVER (Insect and Nematode Hormone Laboratory, ARS, Beltsville, MD) and JOHN NEAL (Florist and Nursery Crops Laboratory, ARS, Beltsville, MD).

Many insect species have developed chemical defenses against avian predators. Here, we present a series of behavioral investigations designed to assess the repellency of chemicals secreted by nymphs of the azalea lace bug (Stephanitis pyriodies). In initial behavioral experiments, we showed that nymphs were strongly avoided by both red-winged blackbirds (Agelaius phoeniceus) and European starlings (Sturnus vulgaris). Adult insects (which lack chemical secretions), were relatively more palatable. To test the hypothesis that adults were eaten because they lacked repellent, we treated adult lace bugs, as well as highly preferred green peach aphids, with the 2 principle components (a hydroxychromone and a diketone) in nymph secretions. In 2-choice tests, treated insects were strongly avoided. Also, aphids and adult lace bugs not treated with the mixture but presented along-side treated conspecifics were avoided, suggesting that the birds were unable to discriminate treated 'model' insects from untreated 'mimics'. We now plan to test individual mixture components to assess whether all are required for repellent effects. Lace bug repellents (as well as the offensive secretions of other insects) may represent new and effective tools in wildlife management and animal damage control.

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Seasonal Shifts in Odor Responding by Starlings Are Related to Breeding Behavior. LARRY CLARK (Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104), CYNTHIA A. SMERASKI (Dept. Biology, Bryn Mawr College, Bryn Mawr, PA).*

A passerine bird, the European starling Sturnus vulgaris demonstrated a seasonal ability to respond to olfactory cues. Cardiac conditioned responses were most evident when birds were in breeding condition. Once birds became photorefractory, responding to odors all but ceased. For birds in breeding condition, threshold sensitivity to the odor of cyclohexanone was comparable to levels reported for non-passerine birds (0.3% vapor saturation). In contrast, threshold levels during the late summer and fall, when birds were in a non-breeding state, were 10% vapor saturation; levels high enough to implicate the trigeminal system as the sensory system mediating chemosensory perception. The strong cyclic responding pattern suggests links between photoperiodically controlled endocrine production, breeding behavior, and olfactory sensitivity. Such physiological links are currently being investigated. Behaviorally, the seasonal expression of olfactory function may be advantageous in the exploitation of chemical cues originating from nest material. The avian nest protection hypothesis explains the widespread behavior of incorporating fresh green leaves into an otherwise dry nest matrix as an evolutionary adaptation to counteract the selective pressure of parasites and pathogens. Possessing a capacity to sense the metabolites contained within particular plants could potentially allow starlings to maximize the probability of selecting plants with high biological activity.

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Assessment of Complex Taste of Aspartame and Saccharin in Rats. CHARLES N. STEWART and MARCUS W. THOMSEN (Departments of Psychology and Chemistry, Franklin & Marshall College, Lancaster PA 17604-3003).

Analysis of the taste characteristics of complex substances with rats has traditionally been approached by establishing a conditioned taste aversion (CTA) to the substance and then looking for the generalization of the CTA to specific tastants such a sucrose, quinine, NaCl and HCl. We have coupled this methodology with the effects of pre-exposure to one of the elements of a complex tastant (Na-saccharin) using a latent inhibition paradigm. The results show that pre-exposure to sucrose weakens the CTA to the "sweet" component of saccharin but leaves a heretofore unreported aversion to the Na ion present in this substance. With aspartame as the conditioned stimulus a similar effect is found. When neophobia towards Na saccharin is examined as a function of pre-exposure to the standard tastants only sucrose had a clear effect on the strength of neophobia displayed. Aspartame, unlike Na saccharin, does not evoke strong neophobia in rats even though they readily form a strong CTA to this substance.

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The Effects of Streptozotocin-Induced Diabetes in Rats on Water, Sucrose and Sodium Saccharin Ingestion. JAMES C. SMITH and KIMBERLEY S. GANNON (Florida State University)

Fourteen SD rats were given a single IP or IV injection of streptozotocin (SZ), six rats were given three low-dosage IP SZ injections once a week for three weeks and six rats were given saline injections. Blood glucose levels were measured 24 hr. after injections, and approximately every seven days thereafter. Eight rats (six IP injected and two shams) were placed in special cages where all licks on either of two drinking tubes and entries into a food chamber were recorded as breaks in IR beams. All ingestion activity was routed to a microcomputer and appropriate software allowed for detailed analysis of eating and drinking bouts. Ingestive behavior was recorded when only food and water were present and also when sodium saccharin or sucrose was available in the second drinking tube. The remainder of the rats were housed in standard cages and only daily food and fluid intake were measured. The blood glucose levels for all of the SZ injected rats were above 300mg/dl. Both food and water intake by the SZ rats increased and results of pattern analyses showed a greater incidence of daytime bouts and an increase in bout size. During the first 24 hours with 0.1% saccharin, the SZ rats showed a marked saccharin preference. By the fifth day of testing, the preference dropped to 0.50, but the absolute intake of saccharin exceeded that of the control rats. Sucrose intake at the lower concentrations was high, however, at concentrations of 0.5 M and above consumption by the SZ rats was lower than that for controls. A description of sugar ingestion patterns is presented.

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Taste Responses to Ethylene and Propylene Glycol in Rats and Dogs. DAVID A. MARSHALL (Department of Physiology) and RICHARD L. DOTY (Smell and Taste Center, Department of Otorhinolaryngology and Human Communication, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104).

Although it is widely believed that ethylene glycol (EG) is an attractive tastant for a number of animals, empirical data on this point are lacking. In this series of studies we evaluated EG drinking behaviors of rats and dogs. In Experiment 1, the tendency of 241 dogs to approach and lick a 50% water-based solution of EG was examined. Although the majority of the animals approached the EG, only 12% initiated drinking responses. In Experiment 2, rats and gastric-canulated dogs displayed equivalent rank order preferences for aqueous solutions of 20% sucrose (S), 30% EG, 30% propylene glycol (PG), and water (W) (S > W > EG > PG). Both species evidenced an inverse relationship between EG concentration and amount consumed. Interestingly, the propensity of dogs to ingest EG was not increased by either 23- or 40-hr food and water deprivation. In Experiment 3, the elimination or attenuation of olfactory input in rats (by either olfactory bulbectomy or intranasal zinc sulfate lavage) produced no significant changes in their ingestive responses to EG. In Experiment 4, rats given the opportunity to ingest EG over a 14 hour period developed an apparent taste aversion to this material. Overall, these studies demonstrate that EG ingestion (a) occurs in only a small proportion of animals, (b) is similar in dogs and rats, and (c) is not markedly influenced by the elimination or attenuation of olfactory input.

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Behavioral Methods for the Study of Taste and Ingestion in Mice. KIMBERLEY S. GANNON, JAMES C. SMITH and RACHEL KELLEY (Florida State University)

Two methods used in our laboratory to investigate rat ingestive behaviors have been modified for use with mice. SWR/J and C57BL/6J inbred mice were tested in a short-term procedure with an ascending sucrose series (0.01 M - 1.3 M). Animals were tested with a different sucrose concentration on each of six consecutive days. A single drinking spout was presented to each animal for ten 10-sec. trials and withdrawn for 10 sec. between trials in an effort to reduce postingestional influences. Licks were monitored through electrical contacts which were transmitted to a microcomputer. Mean number of licks/10 sec. increased as sucrose concentration increased from 0.01 M to 0.32 M and remained constant from 0.32 M to 1.3 M sucrose. Strain differences were apparent at higher concentrations with SWR/J mice exhibiting greater lick rates than C57BL/6J mice. To explore food, water and sucrose (0.03 M - 1.0 M) ingestion among mice over an extended period of time, SWR/J and C57BL/6J inbreds were monitored continuously for 23 hrs. each day. Each cage was modified to accommodate a food jar and two inverted, graduated cylinders with drinking spouts. Individual licks were recorded using electrical contact circuits. The amount of time spent in the food chamber was determined from interruption of an infrared light beam. Contacts and beam breaks were transmitted to a microcomputer for subsequent quantification and pattern analyses. Detailed descriptions of food, water and sucrose ingestion patterns for SWR/J and C57BL/6J mice will be presented.

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Cardiovascular Regulation in Rats with Lesions of the Area Postrema. ROBERT J. CONTRERAS & RACHEL HUNT (University of Alabama, Department of Psychology, Birmingham, Alabama)*

The area postrema (AP) is a brainstem structure located in the caudal medulla adjacent to the fourth ventricle. Lacking a blood-brain barrier, the AP is accessible to circulating hormones (such as angiotensin II) that regulate arterial blood pressure. Recent studies in rats with lesions of the AP indicate that the AP may play a direct role in maintaining resting mean arterial blood pressure (MAP) and heart rate (HR), autonomic tone, baroreflex sensitivity (Skoog & Mangiapane, Am. J. Physiol., 254: H963-H969, 1988) and in mediating the pressor effects of chronic angiotensin II infusion (Fink, et al., Hypertension, 9: 355-361, 1987). Lesions of the AP also result in immediate hypophagia and body weight loss that is never recovered. Body weight and blood pressure level are strongly related. However, the studies cited above failed to include a weight-matched sham control group. The purpose of the present study was to determine whether the cardiovascular effects following lesions of the AP are due to the destruction of an important central structure involved in cardiovascular regulation or are secondary to the food intake and body effects of the lesions. In the present study, one group of rats received lesions of the AP, one control group received sham lesions and was fed *ad libitum*, and a second control group received sham lesions and was pair-fed the same amount of food consumed by the lesioned animals. Beginning about 4 wk after recovery from surgery, systolic blood pressure and heart rate were measured weekly for the next 3 wk by tail-cuff plethysmography. MAP and HR responses to the intravenous sequential administration of 40, 80, and 120 ng/kg body weight angiotensin II, 1 mg/kg metoprolol, and 2 mg/kg methyl scopolamine were then obtained from the catheterized femoral artery in awake unrestrained rats. Initial data analysis indicates that the chronic bradycardia seen in rats with lesions of the AP is not due to body weight loss but due to a specific neural deficit associated with the lesion. Additional data analysis of the HR response to metoprolol and scopolamine will help us determine whether the bradycardia is due to decreased sympathetic tone or increased vagal tone, respectively. With additional data analysis, we will also be able to determine whether the pressor effects of peripheral angiotensin II are mediated in part by the AP.

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Preliminary Studies on The Effect of Steroids on Olfactory Function of the Dog PATRICK I. EZEH (Auburn University)
LAWRENCE J. MYERS (Auburn University)

The effects of three steroids (dexamethasone, hydrocortisone and deoxycorticosterone) on the olfactory function of the dog were investigated utilizing 15 clinically normal mixed breed dogs. The functional status of the adrenal glands was tested using the ACTH stimulation test. The olfactory function of the dogs was evaluated by electroencephalographic and behavioral olfactometry techniques. Normal olfactory threshold values were obtained prior to the initiation of the treatment using the response of the dogs to 14 concentrations of eugenol and benzaldehyde, diluted in propylene glycol. Olfactory of the dogs was evaluated during subsequent treatment with steroids. Cortisol level in the plasma was monitored throughout the study period. At the termination of the 4 weeks treatment and subsequent euthanasia of the dogs, the ethmoid tissue was sectioned at 6-7 microns, stained (Meyers' Method) and histologically evaluated. From the preliminary data collected, the steroid used in this study appears to effect the functional status of the olfactory system, elevating the olfactory threshold values to eugenol and/or benzaldehyde measured by the electroencephalographic and innate behavioral response without tissue alterations visible in hematoxylin and eosin sections.

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Regulatory factors in the vertebrate olfactory mucosa. THOMAS V. GETCHELL & MARILYN L. GETCHELL, Dept. of Anatomy & Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201.

Access and clearance of ligands to and from binding sites on olfactory cilia are regulated by a complex interplay of molecular, physical and cellular factors. Nasal/olfactory glands secrete mucus that contains many proteins, among them odorant binding proteins (OBPs) that may solubilize lipophilic odorants in the aqueous mucus phase and subsequently tranport them to receptor sites. The rate of transport of the ligand-OBP complex or unbound odorant is a function of the diffusion coefficient that, under physiological conditions, is determined largely by the molecular size of the complex or unbound odorant and the viscosity of mucus as well as the tortuosity factor. If bound to OBPs, binding constants must favor association of the ligand to the binding protein, dissociation of the complex and re-binding of the ligand to the odorant receptor. The effects that enzymes, such as oxidases, esterases and cytochrome P-450 monooxygenases, may have on this process are not well understood. Extrinsic autonomic (adrenergic, cholinergic) and peptidergic (substance P/CGRP, VIP, LHRH) neurons innervate olfactory glands and regulate both secretory granule release and electrolyte/water balance. Upon secretion, the secretory products presumably regulate properties of olfactory mucus that affect ligand access and clearance, including thickness, sol-gel phase transitions and viscosity. Extrinsic peptidergic (substance P/CGRP, VIP, LHRH) neurons terminate near the epithelial surface in close apposition to sustentacular cells and olfactory receptor neurons. The substance P/CGRP fibers, in addition to functioning as sensory fibers, appear to regulate secretion from sustentacular cells through a secretomotor reflex and to neuromodulate the sensitivity of olfactory receptor neurons to odorant stimulation. The action of regulatory factors in the olfactory mucosa is an emerging topic of research focused on molecular, physical and cellular factors that affect sensory transduction.

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New aspects of the odorant-binding protein: pharmacology and kinetics. JONATHAN PEVSNER, VIVIAN HOU, SOLOMON H. SNYDER (Dept. of Neuroscience, Johns Hopkins School of Medicine, Baltimore, Md.).

The odorant-binding protein (OBP) is an abundant, soluble protein of 18 kDa present in the nasal epithelium of several species. The amino acid sequences of rat and bovine OBP are homologous to $\alpha_{2\mu}$-globulin and a family of ligand-binding carrier proteins. This suggests that the function of OBP could be to transport odorants to (or from) sensory neurons in the olfactory epithelium. The localization of OBP and its mRNA to mucus-secreting glands in the cow and rat is consistent with this proposed function. We have now studied the pharmacology and kinetics of odorant binding to purified bovine OBP. We measured the potencies of 50 odorants in displacing [3H]3,7-dimethyloctanol (DMO) from OBP. Odorants of many structural classes are potent (IC_{50} < 1 μM) including monoterpenes, musks and mercaptans. However, some of the least potent odorants (IC_{50} > 50 μM) are from the same classes. Within a class of odorants, more hydrophobic molecules tend to bind OBP more tightly. We also examined the kinetics of odorant binding to bovine OBP. In equilibrium binding studies, [3H]DMO binds with K_d = 0.3 μM. Scatchard plots are curvilinear, consistent with the presence of two binding sites or negative cooperative interactions between the two subunits of OBP. The association is half-maximal in 10 minutes, and the association rate constant k_{+1} = 5 x 10^6 M^{-1} min^{-1}. The dissociation rate is slow when measured by the method of "infinite" dilution, but this rate is highly accelerated by "infinite" dilution in the presence of excess unlabelled odorant. This suggests that the binding of odorants to OBP exhibits strong negative cooperativity as seen in the binding of insulin to its receptor. For OBP, similar kinetic and equilibrium binding results were observed with the odorant [3H]2-isobutyl-3-methoxypyrazine. The physiological significance of the negative cooperativity is unclear.

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Odorant binding proteins in vertebrates and insects: similarities and possible common function. PAOLO PELOSI & ROSARIO MAIDA, Instituto di Industrie Agrarie - via S. Michele, 56100 Pisa - Italy.

The olfactory system seems to make use of at least three types of proteins: specific receptors, enzymes and binding proteins. Besides being present in the nose all three structures are believed to take part in odor detection and recognition. While no protein has been unambiguously identified as an olfactory receptors, enzymes and binding proteins more or less specific for odorants have been detected and, in some cases, purified, but their role in olfaction is still not clear.

On the basis of the data recently accumulated in the literature, hypothesis can be formulated on the function of such proteins and strategies can be devised to prove or discard them. Particularly informative is a comparison with the pheromone detection system in insects, that bears striking similarities to the vertebrate olfactory system.

Both the bovine OBP (odorant binding protein) and the PBP (pheromone binding protein) from Antherea polyphemus, the two best studied proteins of this class in vertebrates and insects respectively, are dimers of identical subunits of low molecular weight and both bind one molecule of substrate per dimer. Their isoelectric points are very close and they bind odorants or the pheromone with a strength and a specificity lower than what expected for the corresponding olfactory receptors; finally, they are both soluble proteins and very abundant in the organ of olfaction.

Other odorant binding proteins of vertebrates include those isolated from rat and frog, while pheromone binding proteins have been also purified from Lymanthria dispar and Bombyx mori; the data relative to the latter species are presented here for the first time.

Possible functions of these proteins are discussed on the basis of both ligand specificity and tissue localization, with particular reference to insects, whose less complex system provides a model for olfaction in vertebrates.

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Aphrodisin: Pheromone or Transducer? ALAN G. SINGER (New York College of Osteopathic Medicine, Old Westbury, NY 11568) and FOTEOS MACRIDES (Worcester Foundation, Shrewsbury, MA 01545)

Aphrodisin, the major soluble protein in hamster vaginal discharge, stimulates copulatory behavior in male hamsters. It is detected by receptors within the vomeronasal organ of the male. From the loss of behavioral activity after degradation of the protein with heat or proteolytic enzymes, it is clear that the polypeptide chain is an essential part of the pheromone. And from attempts to remove small molecules from the protein there is no evidence for the presence of a transported ligand. However, the abundance, size, charge, and the amino acid sequence of aphrodisin all indicate that it is a member of the recently recognized alpha-2μ-globulin superfamily of extracellular proteins, some of which, such as serum retinol-binding protein and odorant-binding protein, are known to bind small molecules. Consideration of the chemical and biological properties of these proteins, as well as the results of our studies with the pheromonal properties of another alpha-2μ-globulin from female mouse urine, the major urinary protein, both suggest some interesting possibilities for the mechanism of action of the pheromones.

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A model olfactory system: Pheromone detection by moths. MICHAEL R. LERNER, TUNDE K. GYORGYI, JEFFERY REAGAN, ROBERT RYBCZYNSKI & RICHARD G. VOGT, Yale/Howard Hughes Medical Institute, New Haven, Connecticut 06510

How do pheromone molecules reach receptors that are embedded in the dendritic membranes of olfactory neurons and once they have activated receptors, how is their activity curtailed?

While pheromones are extremely hydrophobic, the dendritic membranes are surrounded by a protective aqueous fluid called the receptor lymph. The most popular hypothesis about how pheromone molecules traverse the receptor lymph is that they are ferried by the pheromone binding protein (PBP) which is found only in moth antennae, and in particular, in their olfactory receptor lymph. This water soluble protein is capable of binding pheromone and as a result it can solubilize the hydrophobic chemical into an aqueous environment. We have cloned and sequenced cDNA encoding PBP from M. sexta, determined its amino acid sequence and done a developmental study on the timing of the protein's expression.

Without rapid signal termination, moths may be unable to locate a source of pheromone. We have recently identified an aldehyde oxidase (AOX) in the receptor lymph of M. sexta. Both pheromones in this moth are aldehydes and the AOX prefers pheromone as a substrate over every other aldehyde tested. The developmental profile and biochemical properties of the AOX have been examined. Because of its presence, the half life of pheromone in an antenna in vivo, is probably less than one millisecond. It has also been known for some time that A. polyphemus has an esterase in its pheromone sensitive sensilla and but two pheromones, one of which is an acetate ester while the other is an aldehyde. We have found that in addition to the esterase, males have an AOX in their receptor lymph and are in the process of determining whether in moths a general rule exists: whenever a female produces a pheromone, conspecific males posses an enzyme that degrades it.

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The role of degradative enzymes in chemosensory processes. WILLIAM E. S. CARR. (The Whitney Laboratory, University of Florida, St. Augustine, FL 32086).

After the neurotransmitter acetylcholine is released into a synaptic cleft, its lifetime is regulated by the ectoenzyme acetylcholinesterase which inactivates the signal molecule by hydrolysis. This inactivation process is critical to the signaling system because it limits receptor desensitization and enables the receptor to be reactivated by subsequent pulses of signal. This example of a degradative enzyme limiting the residence time of a signal molecule in internal tissues has apparent parallels in the external chemosensory systems of diverse organisms. In this presentation it will be shown that chemosensory responses are affected by degradative enzymes such as the following which hydrolyze, oxidize, or otherwise transform the structure of chemostimulants: phosphodiesterase and folic acid deaminase and hydrolase in slime molds[1], acetylcholinesterase in protozoans[2], peptidases in yeast[3], esterases in insects[4], nucleotidases in crustaceans[5], and probably monooxygenases and various esterases in mammals[6]. It is believed that these degradative enzymes are examples of perireceptor components that play an important role in many (most?) chemosensory processes by limiting the residence time of stimulants or suppressants in the receptor environment, maintaining receptor sensitivity, decreasing noise levels, producing new stimulants or suppressants, or yielding products affecting other perireceptor components.

[1]McRobbie, CRC Critical Rev.Microbiol.13 (1986). [2]e.g., Doughty, Comp.Physiol.Biochem.61C (1978). [3]Miyakawa et al., J.Bacteriol.169 (1987). [4]Vogt et al. PNAS 82 (1985). [5]Trapido-Rosenthal et al., J.Neurochem.49 (1987). [6]Dahl, in: Margolis and Getchell (eds) Molecular Neurobiology of the Olfactory System, Plenum Press (1988).

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Memory Psychophysics for Smell and Taste. DANIEL ALGOM, LAWRENCE E. MARKS and WILLIAM S. CAIN (John B. Pierce Foundation Laboratory and Yale University, New Haven, CT 06519)

Two experiments used the method of magnitude estimation to assess how perceived and remembered intensities are related to the referent physical concentrations. In the first study, separate groups of subjects made quantitative judgments of taste stimuli (discriminably different, suprathreshold, concentrations of sucrose) either presented physically (perceptual estimation) or represented symbolically (memorial estimation after 24 h). In the second study, subjects made quantitative judgments of odor stimuli (discriminably different, suprathreshold, concentrations of amyl acetate) either perceptually or from memory. Questions of interest included the following: (1) How do remembered odor or taste intensities map onto their referent physical intensities? In particular, (2) Do memory-based psychophysical functions conform to the same mathematical relation -- power transforms -- as do perceptual psychophysical functions? And, (3) if so, do the parameters that govern the functions (i.e., the exponents) remain the same? The data for both taste and smell showed that perceived and remembered intensities related to referent concentration by power functions with similar exponents. The constancy of exponent contrasts with lower memorial exponents reported for other sensory continua as well as with predictions based on theories of memory-based magnitude judgments. The results may imply a unique role for memory in chemosensation.

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Individual Differences in Psychophysical Responses to Chemical Stimuli. ANDREA HOPMEYER and DAVID A. STEVENS (Dept. of Psychology, Clark University, Worcester, MA 01610)

Individual differences are common in psychophysical studies of the chemical senses. This source of variance is typically unaccounted for, and thus contributes to experimental error, increasing the probablility of committing Type II errors in interpreting data. Such errors can be minimized by the recognition and, hence, control of individual differences. Two categorization systems have been useful in this regard in our laboratory. These classify people on the basis of the extent to which they utilize cues from internal sources and actions relative to those from external, situational sources. We have shown for several chemosensory stimui that those people who employ the former cues have steeper psychophysical slopes than do those who principally utilize the latter. The present study examined individual differences in judgments of the intensity of sourness of three solutions of citric acid (.0028, .0056, .012M). The solutions were colored with yellow food dye; the three intensities of color were orthogonal to the concentrations of acid. The subjects, 50 student volunteers, were classified as type of cuer by the extent to which they were influenced by the manipulation of facial muscles (self-produced cues) relative to verbal information (situational cues) in assessing their mood. The results were consistent with the previous work. Self-produced cuers were more sensitive to differences in concentration of acid than situational cuers. The former initially produced a steeper psychological slope for sourness (.74) than the situational cuers (.50).

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Chemosensory Psychophysics and Nutritional Status in Young and Elderly Subjects. MAGDALENA M. GILMORE (Brown University), CLAIRE MURPHY and AUDREY A. SPINDLER (San Diego State University)*

The purpose of the present study was to examine several features of the relationship between chemosensory perception and the nutritional status of young and elderly persons. A total of 46 persons participated: 23 were elderly ($\bar{M}$ = 70.4 ± 5.67 yrs.) and 23 were young ($\bar{M}$ = 23.00 ± 2.20 yrs.). Six members of each age group were male, the remainder were female. All subjects were active, community-dwelling persons who were able to travel to the laboratory for testing. They were paid for their participation. The subjects first participated in a 1 hr training session to learn the procedure for maintaining dietary records and to acquire practice with scales and food models. They were instructed to keep dietary records for three days. At the end of the recording interval, they were interviewed regarding their records and anthropometrics were taken. During the three day recording interval they were required to have one fasting blood assay for protein, albumin and BUN. On the same day that blood was assayed, psychophysical testing was conducted. Using the method of magnitude matching with weights as the calibration continuum, subjects produced intensity estimates for a series of concentrations (0, 1, 2, 3, 4, 5%) of casein hydrolysate, presented in an amino acid-deficient soup base. Stimuli were presented in random order with the presentation of chemosensory stimuli and weights intermingled. Pleasantness of the same series of concentrations of casein hydrolysate in the amino acid-deficient soup base was judged on bipolar line scales, where distance from the midpoint indicated the degree of pleasantness or unpleasantness. Stimuli were again presented in random order. Intensity estimates for casein hydrolysate were significantly related to age and to nutritional status. Dietary intake of protein in this sample of elderly people was high; in fact, the average intake was above the RDA for protein and higher than the average intake of the young subjects. However, serum levels of protein were significantly lower in the elderly subjects than in the young subjects. In fact, the ratio of percent protein in the diet to the serum level of protein was significantly higher in the elderly than in the young subjects. Thus, although they consumed greater amounts of protein, the elderly appeared to absorb less than the young subjects. This tendency may be related to the preference for casein hydrolysate.

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Age-Associated Differences in the Rate of Recovery from Adaptation. CLAIRE MURPHY (San Diego State University),* MAGDALENA M. GILMORE (Brown University), KIMBERLY Y. MALLOY and DIANE BODGE (San Diego State University)

We, as well as many others, have observed age-associated differences in taste function assessed with several psychophysical techniques: thresholds, magnitude matching, and just noticeable differences. Interestingly, these effects appear to be quality-specific, with the bitter quality showing the largest and most consistent effect. There is a considerable literature on adaptation and recovery in the taste system, but to our knowledge, there are no life-span studies on adaptation or recovery in this sensory system. In the present study we sought to determine whether there were age-associated differences in the recovery time after repeated stimulation of the taste system, and whether any observable age-associated differences were quality-specific. A total of 20 subjects participated: 10 were elderly females ($\underline{M}$ = 70.6 ± 3.75 yrs.) and 10 were young females ($\underline{M}$ = 22.6 ± 3.53 yrs.). All subjects were active, community-dwelling persons who traveled to the laboratory for testing. The stimuli were concentration series, prepared in .25 log steps of sucrose (.00032-1 M) and caffeine (.000032-.1M), dissolved in deionized water. Stimuli were presented at room temperature. Thresholds for each subject for each stimulus were determined in a single session, using a two-alternative, forced-choice, up-down staircase procedure (Cornsweet, 1962). The mean of the final five of six reversals determined the threshold for a stimulus. The subject was later adapted to a concentration which was three quarter log steps more concentrated than the step above the threshold for that stimulus. At 30 sec intervals thereafter, the subject was forced to choose which of two solutions was stronger: a blank or her threshold concentration. A criterion of three correct choices in a row was set for determination of recovery. Young subjects recovered more quickly than older subjects after being adapted to caffeine, $p < .05$. The trend was in the same direction for sucrose recovery, but the effect was not statistically significant. The results suggest that the aging process affects the process of recovery from taste stimulation, but that recovery is also dependent on the quality of the stimulus. Since the processes of adaptation and recovery continually influence the sensitivity of the sensory system, their role in age-associated decline in taste function may be very important.

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The Effects of Age and Race on Thresholds and Magnitude Estimates of Edible Gums. SUSAN S. SCHIFFMAN, DEBORAH RASCOE, REYMUNDO A. GARCIA (Department of Psychology, Duke University)

The purpose of this study was to determine the effect of age and race on thresholds and magnitude estimates of edible gums. Twenty two young and twenty four elderly subjects tested five gums (acacia, guar, locust bean, xanthan and algin); half of the subjects were black and half were white. The detection thresholds for young white subjects were: 0.644%, 0.057%, 0.061%, 0.039% and 0.0605% for the gums in the order listed above. The means of the detection thresholds were statistically equivalent as determined by t-test for young whites and young blacks. The thresholds for white elderly subjects were significantly higher: 1.02%, 0.116%, 0.430%, 0.238% and 0.115%. The ratio of detection threshold (elderly)/detection threshold (young) varied with the gum from 1.58 for acacia to 7.05 for locust bean; the average ratio was 3.73. The method of magnitude estimation was used to calculate dose-response curves with the log of the concentration plotted on the abscissa and the log of the perceived intensity plotted on the ordinate. The slopes for young white subjects were: 1.104, 1.059, 0.876, 1.099, and 0.525 for the gums in the order listed. For black subjects, the numeric value of the mean slope was less for all gums; 0.872, 0.939, 0.467, 0.785, and 0.230. For elderly white subjects, the slopes for all gums were less than for young white subjects: 0.407, 0.758, 0.583, 0.882, and 0.379. There is extensive individual variation in the perception of thickeners. In addition, gums can have taste properties such as bitterness in addition to viscosity.

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Interaction of chlorogenic acids with proline-rich proteins: A preliminary objective study of the putative mechanism of astringency. MARGARA NAISH, MICHAEL N. CLIFFORD AND GORDON G. BIRCH. (Department of Food Science and Technology, University of Reading, Whiteknights, PO Box 226, Reading RG6 2AP UK).

The interaction of polyphenols with salivary proteins and glycoproteins is believed to be the cause of the sensation known as astringency. In an attempt to forward the understanding of the mechanism of the astringent response, a systematic study of the interactions between some mono and di-esters of cinnamic acid (known as chlorogenic acids or CGA) and selected proteins is in progress. A number of preliminary results on CGA protein interactions using equilibrium dialysis are presented here, with gelatin as a model for salivary proteins due to its high proline and glycine contents. The CGA are a group of related compounds which differ systematically in structure and conformation, thus making it possible to examine their behaviour on the bases of these differences; also they are substances able to elicit more than one basic taste and some of them are said to be astringent.

The CGAs studied were: 3-Caffeoyl-quinic acid (3-CQA), (4-CQA, 5-CQA, 1,3-di caffeoyl-quinic acid (1,3-di CQA) and 4,5-diCQA. With the exception of 5-CQA, the CGAs were extracted and isolated from coffee beans using preparative HPLC. Characterisation of the CGA was carried out by analytical HPLC and NMR.

When equilibrium dialysis studies are carried out the rate of dialysis at 20°C varies for the individual CGAs, the least hydrophobic dialysing more rapidly in the absence of gelatin. In the presence of gelatin each CGA examined is retarded, but the relative retardation is greatest for 4-CQA and 4,5-di CQA and is not obviously correlated with hydrophobicity alone. Steric factors seem to play an important role in the extent of CQA gelatin interactions.

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Physical and sensory interactions of maltol with sucrose. ALISON F. BINGHAM, JOHN GRIGOR AND GORDON G. BIRCH. (Department of Food Science and Technology, PO Box 226, University of Reading, Whiteknights, Reading, Berks, RG6 2AP, UK).

Threshold values of the enhancer molecule MALTOL (ie 3-hydroxy -2methyl-4-pyrone) are determined for both gustatory and olfactory response. Although the maltol molecule has an apparent specific volume of 0.74 cm^3/g and accordingly possesses an intrinsic bitter taste, it enhances the sweetness of sucrose solutions when tasted either with or without a nose-clip. The enhancement occurs whether the maltol concentration is above the olfactory threshold and below the gustatory threshold (ie 312ppm) or above the gustatory threshold (3000ppm). At supragustatory threshold concentrations of maltol, the persistence of sweetness is more markedly elevated than the intensity of sweetness. Solution property studies clearly show a physical interaction between maltol and sucrose. For example, a positive increment in apparent specific volume of the mixture of maltol and sucrose indicates poor packing characteristics of the sucrose-maltol complex with water molecules. Pmr pulse relaxation studies (T_2 values) show that the marked "water-structure-making" effect of sucrose is largely unaffected by the presence of maltol. However, at low sucrose concentrations (1%) the presence of maltol appears to increase proton exchange rate in the solution and this may be relevant to the accession of sweetener molecules to receptors and their intrinsic activity in the receptor environment.

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Structure-activity studies and solution properties of phenols and phenol carboxyic acids. SARAH E. KEMP, JOHN GRIGOR AND GORDON G. BIRCH (Department of Food Science and Technology, PO Box 226, University of Reading, Berks RG6 2AP, UK).

The apparent specific volumes and pmr pulse relaxation values (T_1 and T_2 values) are compared in a number of phenols and phenol carboxylic acids, in relation to their dissociation constants and tastes. Dissociation constants for phenol carboxylic acids are much greater (10^{-2}-10^{-4}M) than for phenols (10^{-9}-10^{-10}M) but these ranges are insufficient to affect the apparent specific volume values in either set of compounds. Introduction of a carboxyl function to a phenol depresses apparent specific volume from the bitter region (0.72 om^3/g) to the sweet region (0.65-0.70 om^3) and this is accompanied by a general increase in spin-spin relaxation time (T_2 values 2.7-3.4 seconds) presumably as increased proton concentrations disrupt water structure. Spin-lattice relaxation times (T_1 values) do not show such clear distinctions between the two sets of compounds. However, this is entirely predictable from the known constancy of T_1 over a broad pH range in water, the differences between T_1 and T_2 reflecting differing concentrations of non-exchangeable protons. Titratable acidities for the phenol carboxylic acids were within the range 10-14 ml. .01M-NaOH/2g of solute and much greater than those of the phenols. This accounts for the greater frequency of perceptible sourness in the phenol carboxylic acids. However, both sets of compounds have published bitterness and/or sweetness thresholds in most cases and may also elicit astringency. Of greater interest is the time-dependent variation of taste quality which these potentially multi-sapophoric molecules may possess, eg already known for gallic acid (Shamil and Birch, 1989). The sourness of this molecule rapidly declines to leave a low but persistent sweet response. The low T_2 value (3.0 seconds) and apparent specific volume of (0.651cm^3/g) of gallic acid help to explain this phenomenon.

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Carboxylic Acid Replacements in Urea Sweeteners: A New Sweet Taste Inhibitor.

GEORGE MULLER (The NutraSweet Company)
J. CHRIS CULBERSON (The NutraSweet Company)
WILLIAM H. OWENS (The NutraSweet Company)

Three bioisosteric replacements of the carboxylic acid group in the Suosan series of sweeteners were prepared and evaluated for taste properties. The parent compound, N-(4-cyanophenyl)-N'-(3-carboxyethyl)urea, has been reported to have a sweetness potency 450 times that of sucrose. In pharmaceutical research the isosteric replacement of the carboxylic acid group is well documented. N-(4-cyanophenyl)-ureas derived from aminoethylsulfonic acid, aminomethylsulfonic acid, aminomethylphosphonic acid, aminoethyltetrazole, and aminomethyltetrazole were prepared and evaluated. The tetrazole ureas were found to be weakly sweet with poor flavor profiles. The ureas derived from aminoethylsulfonic acid, aminomethylsulfonic acid and aminomethylphosphonic acid were found to be tasteless at 1.0 mg/mL. At 1.0 mg/mL, a compound with a sweetness potency of greater than or equal to 20 times that of sucrose would be identified. The urea derived from aminomethylsulfonic acid was found to competitively inhibit the sweet taste of sucrose.

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A Quantum Chemical Study of Sweet and Anti-Sweet Principles: Conformation and Electrostatic Potential Similarities.

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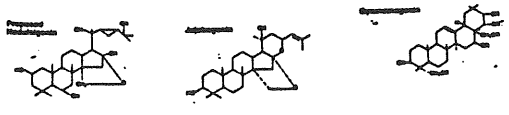
D.J. Gerson (IBM Corp., 1503 LBJ Freeway, Dallas TX)

The stable conformations and electrostatic properties of triterpene sweetness inhibitors (below) have been studied using molecular mechanics and molecular orbital theory methods. The molecules under study consist of a triterpene moiety and an attached gycoside residue. Inorder to reduce the size of the problem to more tractable computational limits for ab-initio electrostatic potential studies, only the triterpene portion of these molecules were explicity considered.

It was found that the most stable conformer of the sweetness reducing agent Hodlucin was approximately 100 Kcal/mol higher in energy (less stable) than the related triterpene glycosides Jujubogenin and Gymnemagenin. Examination of the intramolecular potential for the interconversion amoung the conformers, lead to the assignment of this energy differential to the strain associated with the dihydroxymethyl-cyclopropyl functionality.In the experimental taste perception data reported by Kennedy (1988), Hodlucin exhibited the shortest sweetness inhibition lifetime, while Gymnemagenin the longest. These results correlate well with the computed relative stabilities of the three triterpene molecules. Additionally, a similar analysis was conducted in parallel on four structurally similar sweetners, phyllodulcin, stevioside, glycyrrhizin and dihydrochalcone (DHC). As with the sweetness inhibitors, the longer acting sweetner, DHC, was found to be more stable, by 10 KCal/mol, than stevioside, followed by glycyrrhizin and phyllodulcin. This correlates well with the trend of perceived sweetness intensity for theses molecules. The molecular instability of phyllodulcin, relative to DHC, appears to be related to the magnitude of internal torsional strain, primarily due to a subtituent phenolic residue, than the less conjugated DHC molecule. QSAR analysis, underway, of the electrostatic potential surface and the observed sweetness and/or inhibition should provide further insight into the relationship between the molecular and electronic structure of the agents and their receptor sites.



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Internal Consistency Reliability of the Fractionated and Whole University of Pennsylvania Smell Identification Test. UDAYAN AGRAWAL, RICHARD L. DOTY, and RICHARD E. FRYE (Smell and Taste Center, Department of Otorhinolaryngology and Human Communication, and Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, PA)

The internal consistency reliability (ICR) of the 40-item University of Pennsylvania Smell Identification Test (UPSIT) and its 10-, 20-, and 30-item fractions was explored, as well as the relationships between the fractions and the entire 40-item test. Pearson correlation coefficients (r's) were computed among all independent combinations and permutations of the four 10-item UPSIT booklets using data from 774 subjects. The median r values of the 10- and 20-item combinations were used to establish the ICR's of the 10- and 20-item tests. The ICR's of the 30- and 40-item tests were estimated using the Spearman-Brown formula and the median r's of the 20-item combinations. Additional ICR estimates of the 40-item UPSIT were obtained from non-symmetrical fractions using the Horst formula. The ICR's for the UPSIT and its 10-, 20-, and 30-item fractions were 0.922, 0.752, 0.855 and 0.898, respectively. No major sex differences emerged. Estimates of correlations between (a) single booklets and two-booklet combinations and (b) the 40-item UPSIT using Guilford's (1953) correction for non-independence ranged from 0.812 to 0.871. Overall, these results indicate that (a) the UPSIT and its 10-, 20-, and 30-item fragments have very high ICR's and (b) individual UPSIT booklets or their combinations can be used to assess smell function in a reliable manner where extreme time constraints are present (e.g., in surveys and in brief neuropsychological test batteries).

Supported by Grant NS 16365 from the National Institute of Neurological and Communicative Disorders and Stroke.

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Are there Correlates of Hedonic Estimates in the Power Spectra of the Human EEG? TH. HUMMEL, ST. FORSTER, E. PAULI, and G. KOBAL (Department of Pharmacology and Toxikology, University Erlangen-Nuremberg, Universitätsstr. 22, D-8520 Erlangen, FRG)

The aim of the present study was to find correlates of the hedonic components of olfactory sensations in the human spontaneous electroencephalogram. 80 righthanded volunteers (40 male, 40 female) participated in the experiments. The EEG was recorded from 8 recording sites referenced to linked ear lobes. Each of the 4 odorants cinnamaldehyde, acetaldehyde, menthol, and linalool was applied two times to the left and to the right nostril in a pseudorandomized order. Subjects evaluated: (a) the perceived intensity, (b) the pleasantness/unpleasantness. Females rated linalool and cinnamaldehyde at higher intensities than male subjects ($p<0.05$). Menthol and cinnamaldehyde stimulation effected the highest scores of pleasantness, acetaldehyde was the most unpleasant stimulus ($p<0.01$). Acetaldehyde was less unpleasant to male than to female subjects ($p<0.05$). There were negative correlations between intensity estimates and pleasantness for acetaldehyde and linalool stimuli ($p<0.01$). However, menthol stimulation of the left nostrils revealed a positive correlation between intensity estimates and pleasantness ($p<0.05$). We could not find any correlation between hedonic ratings and the EEG power spectra. Discriminant analysis revealed no differences between power spectra which were obtained from periods classified according to hedonic estimates of the subjects. However, differences between the odorants were found in the alpha-range at frontal and central recording sites ($p<0.05$).

This research was granted by the DFG Ko 812/2-2.

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Impact of Individual Nostril Olfactory Ability on Binasal Olfactory Performance. HORNUNG, D.E., D.A. LEOPOLD, P.R. SHEEHE, M.M. MOZELL, and S.L. YOUNGENTOB (State University of New York, Health Science Center, Syracuse, N.Y. 13210)

To determine the relationship between binasal and uninasal odorant confusion matrix (OCM) scores, we tested five models which, from different theoretical points of view, use the two uninasal scores to predict the binasal percent correct. Some models assumed independent perception via the two nostrils whereas others assumed that the better side determined binasal performance. Additionally, some models predicted binasal scores from the better side scores odorant by odorant, whereas other models predicted binasal scores from the overall better side for all the 10 odorants of the OCM. Binasal and uninasal OCM scores were collected from 64 patients seen in the S.U.N.Y. Clinical Olfactory Research Center. The model in which the higher total uninasal score was used to predict the binasal score had the greatest accuracy, person by person, in predicting the actual binasal scores. This observation suggests that when humans have a "better" overall nostril, it is the one that determines the effectiveness of the overall odorant processing mechanism. As a result, even though the "worse" nostril may be better for a particular odorant, this incremental information tends to be lost when both nostrils are used. In the majority of our patients, the left nostril showed a higher percent correct than did the right nostril (35 had a higher % correct OCM score on the left side, 23 a higher score on the right, and 6 the same score). Since CT scans on a few of these patients showed a larger nasal cavity on the left side, it is possible that uninasal olfactory performance on the OCM may be directly related to the size on the nasal cavity.

Supported by POI NS 19658.

98

EEG and Behavioral Responses to Low-Level Galaxolide Administration. TYLER S. LORIG, ESTHER HUFFMAN, ANTHONY G. DeMARTINO, & JAY DeMARCO (Washington & Lee University).

Previous studies from our laboratory have demonstrated that low, undetected levels of odorant affect EEG and may affect subjective report of mood. The study reported here is a replication and extension of this previous research. Six female and six male undergraduate students participated in the study of EEG and behavioral responses during odorant administration. Galaxolide was the odor chosen for the present investigation because of its rapid habituation and relatively high detection thresholds. In phase 1 of the experiment, odor detection thresholds were established using serial dilutions administered in ascending and descending orders. Subjects were also asked to report odor detections during random exposure to the dilution set, filtered room air and other odors. In phase 2 of the experiment, EEG data from nine electrode locations were recorded during counterbalanced exposure to the serial dilution set and filtered room air. Subjects were exposed to each odor and no-odor (filtered air) condition for 15 seconds with 60 seconds of filtered air between trials. Phase 3 of the experiment involved a computer controlled visual search tasks using letters. In this phase of the experiment, subjects were timed as they attempted to find a target character among a matrix of similar characters while either filtered room air or sub-threshold concentrations of galaxolide were administered. Results of the experiment indicated that EEG alpha activity was reduced during administration of sub-threshold concentrations of galaxolide compared to filtered air ($F(1,11)=18.12$, $p=.0013$). Additionally, performance of the visual search task was impaired during administration of sub-threshold galaxolide compared to the no-odor condition ($F(1,11)=10.17$, $p=.0086$). These results support the hypothesis that undetected odors can alter central nervous system activity and extend this hypothesis to include the alteration of behavior.

The galaxolide was generously donated by IFF.

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Tracing the Central Connections of the IXth Cranial Nerve in the Hamster Using Periganglionic Placement of HRP or Fluorescent Tracers. SHERYL K. BRINING, MICHAEL N. LEHMAN, and DAVID V. SMITH (University of Cincinnati College of Medicine).

Previous studies which have traced the central connections of the IXth cranial nerve (IXn) have required transganglionic transport of the tracer. After application to the distal nerve, horseradish peroxidase (HRP) transported to the central nervous system can be visualized at a light microscopic level using the chromagen tetramethyl benzidine. Electron microscopic (EM) observations require the use of the less sensitive chromagen diaminobenzidine (DAB) and consequently many fewer HRP-labeled fibers can be visualized. We have circumvented this problem by placement of HRP into the region of the petrosal ganglion. Using a ventral approach, the IXn in the hamster was identified lateral and deep to the larynx, a deep dissection was done, part of the tympanic bulla was removed, and the skull base was exposed where the IXn traversed the cranium. The ganglionic portion of the nerve was isolated, partially transected, and crystals of HRP were applied to the nerve every 15 minutes for 2-3 hr. After a 48-hr survival, animals were perfused intracardially with paraformaldehyde/glutaraldehyde fixative. In vibratome sections processed with DAB, many IXn fibers were seen entering the brainstem and coursing into the nucleus tractus solitarius (NTS) and spinal trigeminal nuclei. Within the NTS, fibers and terminal boutons were easily visualized at the light level. We are currently sectioning this material for EM analysis. This surgical approach has also allowed us to investigate the relationship between IXn input and neuropeptides present in the NTS and brainstem. Approximately 25 ul of the fluorescent tracer rhodamine B isothiocyanate (4%; RITC) were placed into the IXn ganglionic region. After 5-10 days survival, the animals were perfused intracardially with 4% paraformaldehyde. Frozen sections through the brainstem were processed for the immunocytochemical detection of substance P (SP) and tyrosine hydroxylase (TH) using an avidin-fluorescein label. RITC-labeled IXn fibers were seen entering the brainstem together with TH- or SP-immunoreactive fibers. Within the NTS, TH-positive cells were intermingled with afferent terminals of RITC-labeled IXn fibers. Substantial SP immunoreactivity was localized more medially in the NTS than the terminal distribution of IXn fibers. Other antigenically identified neurotransmitter substances are currently under investigation.

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Genetic Differences in the Organization of the Rostral Solitary Nucleus in C57 and BALB/c Mice. DIANE L. KACHELE & PHILLIP S. LASITER (Florida Atlantic University).

Murine strains show considerable genetic variability in taste reactivity to various chemical stimuli. Neurological correlates of these differences have not been examined, however. To that end, the present study investigated taste preferences and aversions, size of chorda tympani (CT) terminal fields, and organization of glutamate and GABA immunoreactive neurons in CT terminal fields of the nucleus of the solitary tract (NST) in BALB/c-Crl and C57BL-Crl mice. Results indicate that the two strains differ significantly with regard to behavioral preferences to NaCl and sucrose. BALB/c mice demonstrate higher preferences to 50 and 100 mM NaCl, as compared to C57 mice. C57 mice exhibit greater preferences to 25, 50 and 100 mM sucrose concentrations, as compared to BALB/c mice. No difference between strains has been confirmed for aversions to citric acid or quinine hydrochloride. Preliminary results from peroxidase labeling experiments indicate that BALB/c mice possess a greater number of cells in the Geniculate ganglion that gives rise to axons contained in the CT nerve, as compared to C57 mice, and volumes of CT terminal fields in the NST of BALB/c mice are correspondingly larger than those of C57 mice. Preliminary immunohistochemical analyses indicate that a correspondingly greater number of glutamate-like immunoreactive (GLU-LI) and GABA-LI neurons are contained in CT terminal fields of BALB/c mice, as compared to C57 mice. However, volumetric density of both GLU-LI or GABA-LI neurons is nearly equivalent in CT terminal fields of each strain. These results demonstrate that differences in taste preferences of the BALB/c and C57 mice are stimulus-specific and may be mediated, at least in part, by differences in the number of CT afferents that innervate the anterior tongue, the topographic distribution of CT fibers in the NST, or the total number of NST neurons that project axons to higher-order gustatory relays, particularly the parabrachial gustatory zone.

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Ultrastructure of an Identified Cell Type in the Gustatory Subdivisions of the Nucleus of the Solitary Tract in the Hamster. MARK C. WHITEHEAD (Ohio State University, Columbus, OH).

The rostral central (RC) and rostral lateral (RL) subdivisions of the nucleus of the solitary tract (NST) are the sites where most gustatory primary afferent axons synapse. Additionally, these subdivisions contain elongate cells that have ovoid cell bodies and two proximal dendrites, one oriented medially, the other laterally. Elongate cells, first identified in Golgi material (Whitehead, 1988, JCN, 276:547-572), have been filled by HRP injections at taste-responsive recording sites, and identified as projection neurons by HRP injections in the medial parabrachial nucleus (PBN). In this study of 4 animals, HRP-labelled elongate cells, that project to the PBN, were examined with electron microscopy to define the distributions and types of synaptic endings providing their input. Elongate cell inputs were of three types: endings with dense-cored vesicles synapsing with proximal dendrites, endings with flat or pleomorphic vesicles forming symmetrical junctions with proximal dendrites or cell bodies, and endings with round vesicles forming asymmetrical junctions with proximal dendrites and rarely with cell bodies. The latter two endings resemble SP (possibly interneuronal) and facial primary afferent endings, respectively, that I have previously described. These inputs were sparsely distributed whether the labelled cells were in the RC subdivision or in the RL subdivision among axons of the solitary tract. The possibility that distal dendrites receive denser inputs is under investigation. In summary, elongate cells that project to the pons receive input from a variety of synaptic ending types including, perhaps, primary afferent taste axons.

Supported by NIH Grant NS25708.

103

Cytoarchitecture of the Pontine Taste Area in the Golden Hamster. CHRISTOPHER B.HALSELL and MARION E.FRANK (Center for Neurological Sciences and Dept. of BioStructure & Function, UCONN Health Center, Farmington CT. 06032)

The parabrachial nucleus (PBN) is the obligatory third-order relay for the ascending gustatory system in rodents. Only a part of the PBN, the pontine taste area (PTA), is responsive to taste stimuli. Although the physiology of the PTA has been examined in hamsters, it has not been mapped nor has its cytoarchitecture been characterized. Glass micropipettes where used to map taste-responsive areas of the PBN by recording multi-unit activity and depositing horseradish peroxidase iontophoretically. Taste stimuli (0.03 M NaCl, 0.1 M KCl, 0.1 M sucrose and a mixture of the three) were applied to the anterior tongue. The normal cytoarchitecture of the PBN was examined in 20 and 40 um frozen sections cut in the horizontal, parasagital and coronal planes. The PTA for the anterior tongue is located within the medial and ventral lateral subdivisions of the PBN, which are separated by the superior cerebellar peduncle. The medial subdivision is located throughout the caudal two-thirds of the PBN, whereas the ventral lateral subdivision is only located within the middle third of the PBN. Taste activity was never recorded in the other seven subdivisions of the PBN. The medial subdivision is heterogenous, containing three conspicuous cell types. The large round-multipolar neurons (av. diameter= 11.99 +/- 1.5 um) stain lightly for Nissl substance, but the medium oval-multipolar (av. diameter= 9.02 +/- 1.6 um) and the fusiform neurons (av. diameter= 11.92 +/- 2.5 um) stain darkly. The ventral lateral subdivision contains mostly large oval-multipolar lightly staining cells (av. diameter= 11.88 +/- 1.6 um). These results suggest that there is a well defined area of gustatory function in the PBN, that diverse cell types may be involved in gustatory processing, and provide information required for tract-tracing studies aimed at establishing gustatory, as distinct from visceral, pathways.

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Single-unit Submodality Mapping of Taste Responses in the Nucleus of the Solitary Tract. LAWRENCE D. SAVOY, MARTHA MCPHEETERS and THOMAS P. HETTINGER (Department of BioStructure and Function, University of Connecticut Health Center, Farmington, CT 06032)

Single-unit mapping of the taste-responsive region of the nucleus of the solitary tract (NTS) in the hamster has been attempted with the idea of recording from as many cells as possible in a single animal. Such a study offers a higher spatial and functional resolution than is possible from multiunit recordings and simplifies anatomical reconstruction of the recording sites over that obtained from a collection of single-unit sites in many animals. Glass micropipettes filled with 4% horseradish peroxidase (HRP) in 0.5 M KCl - 0.05 M Tris buffer pH 7.5 having impedance values of 1-2 Mohms were useful in picking up background multiunit activity and also capable of single unit isolation. Locations of recording sites were established in reconstructed serial sections from the positions of HRP deposits at several defined locations outside the recording area. To date we have been able to record serially in the same animal within a region of less than 0.1 mm^3 of the rostral NTS from as many as 7 different neurons responding to taste stimulation of the anterior tongue. Spontaneous rates as well as relative responses and impulse patterns to 0.03 M NaCl, 0.1 M sucrose and 0.1 M KCl varied considerably. This sample size is too small to discern a clear spatial segregation of response properties. Relatively complete maps could probably be obtained by recording from about 20 different units in one NTS. Under favorable circumstances, about 1 unit/hr can be sampled, so that useful single-unit mapping is very difficult, but nevertheless technically feasible.

Supported by NIH grant NS16993.

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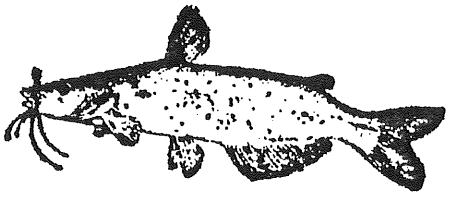

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Gustatory Neural Responses to Polysaccharide and Starch in the Rat

B. K. GIZA (University of Delaware)
R. F. ANTONUCCI (University of Delaware)
A. SCLAFANI (Brooklyn College)
T. R. SCOTT (University of Delaware)

Polycose and amylopectin are highly palatable to the rat. The tastes however, do not generalize well to that of sucrose or of the other prototypical stimuli--NaCl, HCl and quinine--in behavioral tests. We used these chemicals in addition to a standard array of taste stimuli during recordings from 63 single neurons in the NTS of anesthetized rats to determine their neural effectiveness and relative taste qualities. Amylopectin (2%) elicited only a few spikes, always at stimulus onset; Polycose, at 0.1 M, evoked a burst of activity followed by a moderate tonic response. The time courses of both responses were similar to the phasic-tonic sequence of non-sweet stimuli. The profile of activity evoked by Polycose across neurons was most similar to those elicited by Na-Li salts and by quinine; it showed moderate similarity to the patterns representing HCl and MSG and modest correlations with the patterns for sugars. The results of behavioral studies have led to the suggestion that Polycose represents a unique taste quality. Our electrophysiological data align Polycose with all non-sweet chemicals and, to a lesser extent, with sugars as well, implying a complex quality. The basis for its highly appetitive nature is not apparent from the neural response Polycose evokes.

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Neurons in the Cortical Taste Areas of Primates with Preferential Responses to Monosodium Glutamate.

Leslie L. Wiggins and Edmund T. Rolls (Dept. of Exptl. Psychology, University of Oxford, Oxford OX1 3UD, England)

To investigate whether there are neurons in the cortical taste areas of primates which have responses selective for monosodium glutamate, the responses of single neurons to the taste stimuli 1.0 M glucose, 0.1 M NaCl, 0.1 M sodium glutamate, 0.01 M HCl, 0.001 M quinine hydrochloride and water were recorded in the primary (frontal opercular and insular) and secondary (caudolateral orbitofrontal) cortical taste areas in three cynomolgous macaque monkeys. It was found that for 12.7% of the neurons recorded, the best response from the set of 6 taste stimuli was to monosodium glutamate. Moreover, for 8.1% of the neurons recorded, it was found that the response to the taste of glutamate was not correlated with the response to the taste of NaCl, so that these were not simply "salt best" neurons. Further, cluster analyses and multidimensional scaling analyses confirmed that there was more than one type of glutamate-sensitive neuron, at least one of which was primarily sensitive to the taste of glutamate. It is concluded that in the primate there are different populations of neurons which are either primarily responsive to glutamate or combine glutamate sensitivity in different ways with responses to other tastants. This neurophysiological analysis shows that the taste of glutamate is analysed and represented in several different cortical information channels in primates, and that this representation allows for processing which is separable from that to other prototypical tastants.

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Effects of electrical stimulation of the gustatory neocortex on taste-responsive units in the parabrachial pons.

S. MONROE & P.M. DI LORENZO (Dept. of Psychology, SUNY at Binghamton, Binghamton, N.Y. 13901)

Although the gustatory neocortex (GN) sends direct projections to the parabrachial nucleus of the pons (PbN), the GN may also influence the PbN via indirect pathways, through other neural structures. The function of this input in the neural processing of gustatory information remains a mystery. Previous data from our lab (Di Lorenzo, in preparation) has shown that procaine infusion into the ipsilateral GN altered the taste responses in 40 out of 42 (95%) PbN units. The present experiment was designed to investigate the extent of direct GN projections on taste-responsive units in the PbN. Electrical activity was recorded from 38 taste units that were isolatd in the PbN of 13 male Sprague-Dawley rats. Electrical stimulation was applied to the GN with bipolar stimulating electrodes (5, 10, and 15 volts applied at 1, 25, 50 and 100 pps). Units were also tested for responsiveness to chemical stimulation applied to the tongue. Stimuli included NaCl (.1 M), HCl (.01 M), Na-saccharin (.004 M), sucrose (.5 M) and quinineHCl (.01 M). Evoked responses to GN stimulation were observed in 9 of the 38 taste units (26%). Of these, 7 units produced excitatory responses following GN stimulation (mean latency = 13 msec) and 2 units showed evidence of inhibition. No evidence of antidromic activation of PbN units was apparent. PbN units that followed GN stimulation could not be distinguished from those that did not follow either by their spontaneous rates or their response profiles. Although the mean response rates produced by NaCl and HCl in the followers was lower than that of the non-followers, there was considerable overlap. These results suggest that the influence of the GN on the PbN may be mediated primarily via other neural structures or through interneurons within the PbN.

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The Effect of Parabrachial Nucleus Lesions on Taste Aversion Learning in Rats. ALAN C. SPECTOR (University of Pennsylvania), RALPH NORGREN (Hershey Med. Ctr., Penn State Univ.), and HARVEY J. GRILL (University of Pennsylvania).

In an attempt to explore further recent findings suggesting that lesions in the parabrachial nucleus (PBN) disrupt conditioned taste aversion (CTA) formation, a variety of different behavioral measures of aversion were employed. Rats with electrophysiologically-guided lesions in the PBN (PBX, n=15) received 7 intraoral infusions (30 s, 0.5 ml) of 0.1 M sucrose spaced every 5 min beginning immediately after the injection (IP) of 2.0 mEq/kg of either lithium chloride (LiCl) or sodium chloride (NaCl). Oral motor taste reactivity behaviors were videotaped for later analysis. On a subsequent occasion, these rats were treated in a similar fashion as described except that 0.1 M NaCl served as the conditioned taste stimulus. Rats that previously received injections of NaCl were injected with LiCl and vice versa. Preliminary data analysis suggests that LiCl-injected intact control rats (n=15) systematically decreased their ingestive responses while progressively increasing their aversive responses to the taste stimulus during the 30 min. In contrast, many of the LiCl-injected PBX rats did not appear to alter their ingestive or aversive behavior over the course of the session. When histological analysis is completed comparisons between the degree of deficit and the cytoarchitectural lesion parameters will be performed. Experiment 2 employed a different behavioral procedure. On two occasions, these same rats had their respective CTAs strengthened by pairing 15 min access with the taste solution (either NaCl or sucrose) with LiCl injection. These rats had been trained to maintain spout contact for intermittent water reinforcement (70 ul) in a specially designed gustometer. All rats were subsequently tested in this gustometer for their avoidance of 70 ul samples of various concentrations NaCl and sucrose randomly dispersed among water trials. Preliminary analysis indicates that PBX rats showed markedly attenuated levels of avoidance of the LiCl-paired taste as compared to controls.

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Taste Responses of NST Neurons are Altered in Rats "Recovered" From Sodium Deprivation Instituted During Early Development. MARK B. VOGT & DAVID L. HILL (University of Virginia).

Restriction of maternal dietary sodium by embryonic day 8 reduces taste responses in the chorda tympani nerve (CT) to NaCl in the offspring. Responses to non-sodium salt stimuli are unaffected. These effects are not permanent, however, because normal CT responses to sodium salts "recover" when deprived rats are fed a sodium replete diet. The CNS neurons that receive taste input from fibers of the CT are located in the anterior portion of the nucleus of the solitary tract (NST). To learn whether the taste responses of NST neurons are altered by sodium deprivation and recovery, we recorded neurophysiological responses to chemical stimuli flowed over the tongue in rats deprived of dietary sodium from embryonic day 3 to postnatal day 28 and subsequently fed a sodium replete diet for at least 60 days. Preliminary results are based on data from 17 neurons in recovered rats and 13 neurons in adult controls always fed a sodium replete diet. For all non-sodium salt stimuli tested (0.05, 0.1M, 0.5M NH_4Cl, 0.1M $CaCl_2$ and KCl) the mean response frequencies of recovered neurons were slightly less than those of controls. However, for all sodium salts (0.05M, 0.01M, 0.5M NaCl and Na acetate, 0.1M $NaNO_3$), and 1.0M sucrose, the mean response frequencies of recovered neurons were greater than those of controls. Furthermore, a greater proportion of recovered neurons (58%) were "sodium best" responders (0.1M NaCl response frequency at least double 0.1M NH_4Cl response) compared to control neurons (28%). These data suggest that inadequate dietary sodium during early development may result in long-term alterations in NST taste responses that are not ameliorated with the institution of a normal sodium diet. These neurophysiological alterations may relate to other enduring neuroanatomical and behavioral consequences of early sodium restriction that we report at this meeting.

Supported by NIH grants NS24741, NS01215 and MH18411.

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Behavioral Taste Responses in Rats "Recovered" From Sodium Deprivation Instituted During Early Development. DAVID L. HILL & MARK B. VOGT (University of Virginia).

Restriction of maternal dietary sodium on or before embryonic day 8 reduces taste responses in the chorda tympani nerve to NaCl in the offspring. Responses to non-sodium salts and non-salt stimuli are unaffected. These effects in receptor function are not permanent, however, because normal sensitivity to sodium salts "recovers" when deprived rats are fed a sodium replete diet. To learn whether behavioral responses to NaCl are altered by sodium deprivation and recovery, rats deprived of dietary sodium from embryonic day 3 to postnatal day 28 and subsequently fed a sodium replete diet for at least 60 days received two-bottle preference or conditioned taste aversion tests. Compared to rats always fed a sodium replete diet (controls), recovered rats preferred less NaCl (0.005M-0.25M) than controls on two-bottle preference tests. Although the major contributor to the difference in preference can be attributed to larger water intakes in recovered rats, absolute intakes of NaCl were also affected. In contrast to NaCl, responses to NH_4Cl on two-bottle preference tests were similar for control and deprived rats. In order to examine similarities for taste quality perception of salts in recovered and control rats, animals were conditioned to avoid 0.1M or 0.5M NaCl and then tested for generalization to a variety of sodium- and non-sodium salts. Although recovered rats avoided the conditioned stimulus (CS), they avoided it less than controls. Furthermore, they generalized the avoidance of the CS to other stimuli differently than did controls. These findings suggest that behavioral responses of "recovered" rats show enduring alterations as a consequence of early sodium restriction. Permanent central nervous system alterations, which are described at this meeting, may account for the lack of recovery.

Supported by NIH grants NS24741, NS01215 and MH18411.

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Morphology of Neurons in the Anterior Tongue Projection of the Nucleus of the Solitary Tract during Development in Sheep. SUAT GURKAN (School of Dentistry, University of Michigan) and CHARLOTTE MISTRETTA (Schools of Dentistry and Nursing, University of Michigan, Ann Arbor, MI 48109)

From past studies of receptive field size and response characteristics of second order taste neurons in the nucleus of the solitary tract (NST), we know that there is an extensive and increasing convergence of first order, chorda tympani nerve afferents onto NST neurons in sheep. Accompanying this developmental convergence is a general increase in response frequency to various salt stimuli and an acquisition of response properties that distinguish among NST neurons. We have begun studies to discern the morphology of NST neurons in sheep during developmental periods of increasing convergence and changing salt taste response properties. Sheep aged 85 days of gestation (term = 147 days) through 60 days postnatal have been used. In combination with experiments to record salt taste responses and subsequently place lesions in the NST, we are using Nissl, Golgi - Cox and succinate dehydrogenase stains to locate regions of the NST that have characteristic response properties. Golgi - impregnated neurons from coronal and horizontal sections of the brainstem are reconstructed and are being compared across age groups. It is apparent that the dimensions of the anterior tongue projection region of the NST and density of cells alter during development. Various morphological cell types have been observed across developmental stages and descriptions of possible changes in proportions of different types are in progress. These studies to correlate structural and specific functional properties of neurons will contribute to understanding the microorganization of taste regions of the NST.

(Supported by NIH Grant NS25825.)

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Effects of Early Postnatal Receptor Damage on Development of the Rostral Solitary Nucleus in Rat. PHILLIP S. LASITER & DIANE L. KACHELE (Florida Atlantic University).

Normal development of the rostral gustatory zone of the nucleus of the solitary tract (NST) occurs in a progressive manner during postnatal life. Specifically, our previous studies have shown that volumetric density of presynaptic terminals increases from approximately postnatal day 1 (P1) to approximately P10. Dendritic growth occurs for all morphological categories of rostral NST neurons between the ages of P6 and P20. On the basis of these findings we wished to determine whether or not temporally-sequential patterns of pre- and postsynaptic development in the NST are dependent upon normal afference in lower-order (peripheral) gustatory pathways. Rats aged P2 received unilateral superficial cauterizations of the dorsal, lateral, and ventral tip surfaces of the anterior tongue to the level of the intermolar eminence. Beginning 5 days after tongue damage animals were sacrificed and NADH-dehydrogenase (NADH-DH) histochemistry was performed. NADH-DH histochemistry was performed because we have demonstrated that elevated NADH-DH activity reliably identifies the CT terminal field in the NST of normal rats. Dramatic differences were obtained in the volumes of NADH-DH reactions following unilateral receptor damage. From 5 days to 8 days following damage, volumes of NADH-DH reactions were essentially similar ipsilateral and contralateral to damage. Beginning at approximately 8 days following damage to 23 days following damage normal volumetric development of the CT field was reduced approximately 50% in the NST ipsilateral to damage. Attenuated volumes of NADH-DH reactions persisted to the oldest age group that has been examined (P25), indicating that receptor damage produces relatively permanent reductions in the size of CT terminal fields. This effect appears to be subject to a sensitive or critical period, because unilateral tongue damage produced at P30 did not alter volumes of NADH-DH reactions in the rostral NST. These results indicate that intact gustatory receptors are essential for normal development of the CT terminal field.

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Alterations of Chorda Tympani Projection Areas in the NTS of Sodium Deprived and "Recovered" Rats. CAMILLE TESSITORE KING & DAVID L. HILL (University of Virginia)

Recordings from the rat chorda tympani (CT) nerve have demonstrated that sodium deprivation begun early in prenatal development reliably produces a suppressed taste response only to NaCl. Deprived rats which are fed a sodium replete diet in adulthood for at least 60 days exhibit normal CT responses to all stimuli. In order to investigate whether the changes of the peripheral response are anatomically reflected in the CNS, we examined the projection of the CT within the NTS in Na replete (controls), deprived, and "recovered" animals. HRP was applied to cut CT nerves and the tissue was processed after 24 hours survival. Results indicate that the normal projection area is confined in a discrete ovoid configuration in the rostro-lateral NTS, with the most dense label found in the intermediate zone of the field. On the contrary, the fields of the Na deprived and "recovered" animals are more diffuse, extending into more medial and caudal NTS, with most label located in the dorsal zone of the field. Interestingly, the total projection area is similar in deprived and controls while the total area of the NTS is smaller in the deprived animals, suggesting a reorganization of the field within the NTS. The total projection area in the "recovered' animals, however, is 3 times that of control and deprived animals. This may relate to an increase in growth of the NTS following institution of the replete diet, while the pattern of the deprivation-induced effects on CT projection areas remains unchanged. These findings indicate 1) sodium deprivation may establish unique long lasting interactions between the "taste" area of the NTS and more caudal/medial areas involved in sodium regulation; and 2) the plasticity which is found in the periphery is not reflected in the central projection areas of the gustatory system.

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The Effects of Gustatory Stimulation on Neurons in and Adjacent to the Hypoglossal Nucleus. J.B. TRAVERS and L.M. JACKSON, (Ohio State University, Columbus, OH 43210)

Anatomical studies in both rat (Travers & Norgren, '83) and hamster (Travers, '88) indicate the lack of direct projections between the anterior, gustatory-responsive region of the nucleus of the solitary tract and oro-motor nuclei including the hypoglossal nucleus (mXII). The reticular formation lateral to mXII, however, is implicated as a potential locus of interneurons in gustatory-motor pathways. In the present study, neurons both within and adjacent to mXII were recorded in response to gustatory stimulation in the awake, freely moving rat. Single-cell activity was recorded with fine-wire electrodes simultaneous with recording from a subset of oro-pharyngeal musculature. The majority of cells within mXII (n=11) and immediately adjacent to mXII (n=6) were not spontaneously active but responded with a rhythmic discharge to the intra-oral infusion of water (W) or midrange concentrations of NaCl (N) or sucrose (S). A cross-correlation analysis with the anterior digastric (AD: jaw-opener) allowed classification of cells as either in phase with the AD (tongue protruders) or out-of-phase (tongue retractors). The number of action potentials per lick cycle varied only minimally between water and the gustatory stimuli in these 17 cells. Two additional cells, one within mXII and one adjacent to mXII were spontaneously active but otherwise similar to these 17 neurons. Two cells lateral to mXII, however, showed gustatory responses, i.e. both cells responded with more action potentials per lick to N and S than to W, and both cells showed a response decrement to QHCl. Further, they were not tightly coupled to the rhythmic lick cycle. One cell responded at, or prior to the onset of licking, the second started considerably after the onset of licking. These gustatory-responsive neurons were in a location that overlaps the distribution of reticular projections to the hypoglossal nucleus and other oro-motor nuclei, and therefore provide a potential substrate for gustatory modulation of oro-motor responses.

Supported by NS 24889

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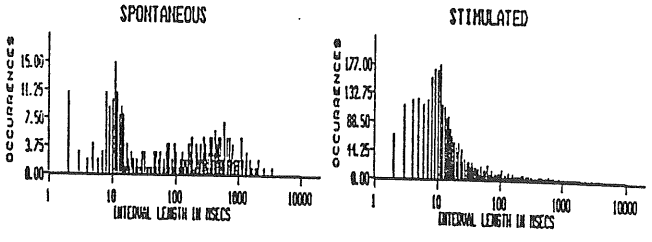
Interspike Interval Patterns : A Method for Classifying Gustatory Central Neurons. SARAH C. NUDING, MARTHA MCPHEETERS & MARION E. FRANK (Center for Neurological Sciences and Dept. BioStructure and Function; UCONN Health Center).

Eighteen taste-responsive single units in the hamster solitary nucleus were isolated and recorded during spontaneous and taste-stimulated periods as described previously (McPheeters et.al. 1988). We calculated interspike intervals (ISIs) from 10 second samples of spontaneous activity (at least 12 trials per unit) and plotted them on a semilogarithmic plot. Four different patterns were evident. Three were based on the graph's shape: bimodal (5 units), unimodal (4 units), and flat (4 units). The fourth was based on a low spontaneous rate (5 units). ISIs were also generated following stimulation with NaCl, KCl, sucrose or a mixture of all three. Ten units changed their ISI pattern in response to stimulation, whereas eight remained constant in shape. This change or consistancy of ISI pattern was characteristic for a unit's responses to all stimuli. Notably, all the units with bimodal ISIs generated from spontaneous activity are classified as NaCl-best units. An example of a bimodal unit which changed ISI pattern following stimulation is shown below.



Supported by NIH grant NS16993.

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Golgi Studies of the Gustatory Zone of the Parabrachial Complex of the Pons in the Hamster. BARRY J. DAVIS (University of Alabama at Birmingham)

The Rapid Golgi method was used to study the typologies of neurons within the so-called "pontine taste area" (PTA) of the parabrachial complex. The PTA is an obligatory synapse in the ascending taste pathway and is identified after the anterograde transport of tritiated amino acids injected into the gustatory zone of the nucleus of the solitary tract (NST). The morphology of pontine neurons have been compared to those of the gustatory NST. As is the case in the NST, pontine neurons are grouped into two broad classes that are defined by the numbers and spread of dendritic branches. The most frequently encountered neuron in the PTA is multipolar and averages 231 μm^2 (14 x 22 μm; N=31). Such neurons are larger than any gustatory NST neuron. Fusiform neurons in the PTA are less common and average 204 μm^2 (12 x 23 μm; N=11), which is similar in size to the fusiform neurons of the gustatory NST. In the gustatory NST, primary, secondary and tertiary dendritic branches account for 91-93% of all dendrites; in the PTA, such branches account for only 76-84% of all dendrites. The remaining higher order dendritic branches possess more complicated branching patterns. The dendrites of PTA neurons also tend to be longer than NST neurons. The dendrites of PTA and NST neurons extend outside their respective cytoarchitectonic boundaries and are generally spine-poor. A major difference between PTA and NST neurons is that PTA neurons (or ascending and/or descending afferent inputs) do not exhibit a preferred orientation in the horizontal plane. Dendritic arborizations demonstrate a range of radial or planar orientations when viewed coronally or sagittally after 3-dimensional rotation analyses. Distinct horizontal tiers of dendritic processes that characterize the gustatory NST are not observed.

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Distribution of Putative Amino Acid Transmitters in the Lamb Solitary Nucleus. ROBERT D. SWEAZEY AND ROBERT M. BRADLEY Dept. of Biologic and Materials Sciences, University of Michigan.

Using the ABC immunohistochemical technique on thick and semi-thin brainstem sections, we investigated the distribution of GABA, glycine, aspartate and glutamate in regions of the lamb nucleus tractus solitarius (NTS) that receive chemosensory inputs from taste buds located at the entrance to the upper airway. In the medial and ventromedial NTS where responses to chemical stimulation of the epiglottis can be recorded, we found numerous immunoreactive *fibers* and *puncta* after incubation of tissue in antisera to each of the amino acid transmitters. In most cases puncta, which are indicative of axon terminals, surrounded non-immunoreactive neurons. Only small numbers of immunoreactive *soma* were observed in the medial and ventromedial NTS after incubation in antisera to GABA and glycine. Immunoreactive soma in the medial and ventromedial NTS were observed more frequently when tissue was processed for glutamate or aspartate than for GABA and glycine; however, overall immunoreactive soma in this region were relatively sparse. The ventral and ventrolateral NTS, where responses to chemical stimulation of the epiglottis are often coordinated with respiration, are areas of the NTS where aspartate and glutamate immunoreactive soma were most abundant, whereas GABA and glycine immunoreactive neurons were most numerous in ventrolateral NTS only. Furthermore, in contrast to NTS areas medial to the solitary tract, immunoreactive puncta were observed adjacent to immunoreactive soma throughout the ventral and ventrolateral NTS. Smaller numbers of immunoreactive soma and puncta were observed in areas of the NTS dorsolateral or dorsomedial to the solitary tract and little immunoreactivity was observed in the subnucleus gelatinosus.

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Convergence of Taste, Olfactory and Visual Inputs in the Orbitofrontal Cortex of Primates.
Edmund T. ROLLS and Leslie L. WIGGINS (Dept. of Exptl. Psychology, University of Oxford, Oxford OX1 3UD, England)

It has been found that there is a secondary cortical taste area in the caudolateral orbitofrontal cortex of the primate (Macaca fascicularis) (Rolls et al., 1985 Chem. Senses 10: 442). This area receives projections from the primary taste cortex in the frontal operculum and insula (Wiggins et al., 1987 Chem. Senses 12: 206). In this study we investigated whether the neurons in this area, and in the more medial caudal orbitofrontal cortex (OFC) which also contains taste responsive neurons (Thorpe et al., 1983 Exp. Brain Res. 49: 93), are multimodal, and receive visual and/or olfactory inputs as well as gustatory inputs. Of the single neurons which responded to any of these modalities, many were unimodal (taste 47%, olfactory 12%, visual 10%), but were found in close proximity to each other. Some single neurons showed convergence, responding for example to taste and visual inputs (17%), taste and olfactory inputs (10%), and olfactory and visual inputs (4%). Some of these multimodal single neurons had corresponding sensitivities in the two modalities, in that they responded best to sweet tastes (e.g. 1M glucose), and responded more in a visual discrimination task to the visual stimulus which signified sweet fruit juice than to that which signified saline; or responded to sweet taste, and in an olfactory discrimination task to fruit odor. These results show that there are regions in the orbitofrontal cortex of primates where the sensory modalities of taste, vision, and olfaction converge; and that in many cases the neurons have corresponding sensitivities across modalities.

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Specific Supression of Glutamate-sensitive Gustatory Neurons of the Shore Crab by L-Aspartate
MANFRED SCHMIDT (Zoologisches Institut, Johann Wolfgang Goethe-Universität, Frankfurt/M.)

The largest population of chemosensory receptor cells in the funnel-canal organs, gustatory sensilla of the shore crab, *Carcinus maenas*, is narrowly tuned to L-glutamate. Only some of these cells respond also to L-glutamine but with a much lower sensitivity. K_M, the concentration causing a half-maximal response is about 10^{-4}M for L-glutamate. In pre-adaptation experiments (with 5 of these cells) L-aspartate was identified as a strong and specific inhibitor of the response to L-glutamate. L-asparagine and L-glutamine had a much weaker suppressive effect, whereas L-α-aminoadipiate slightly enhanced the response. Stimulation with mixtures of L-glutamate and L-aspartate showed that the dose-response curves were shifted to higher L-glutamate concentrations when L-aspartate was present in increasing concentration (10^{-5} to 10^{-3}M). Since the slope of the dose-response curves and the saturation level remained unchanged, the suppressive effect of L-aspartate can be interpreted as competitive antagonism. A Schild plot of the data derived from the dose-response curves gives a linear correlation with a slope close to 0.5 (0.54), and an intersection with the x-axis which corresponds to an inhibitor constant K_I of 1.2 x 10^{-6}M for L-aspartate. This indicates a higher affinity of the L-glutamate receptors for the antagonist L-aspartate than for the agonist L-glutamate (binding constant about 10^{-4}M).

Thanks are due to Dr. W. Gnatzy who supervised this study. It was financially supported by the Deutsche Forschungsgemeinschaft (SFB 45/A1).

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Receptors for Arginine Carboxyl Terminal Peptides: Evidence for their Similarity to the Catalytic Site of Trypsin. D. RITTSCHOF, R.B. FORWARD, JR. (Duke University Marine Laboratory, Pivers Island, Beaufort, NC USA) and B.W. ERICKSON (Chemistry Department, University of North Carolina, Chapel Hill, NC USA).

A relationship between the binding site of a peptide receptor and the catalytic site trypsin is postulated. The difference may be substitution of a basic amino acid for the catalytic site serine. The model is based upon studies of brachyuran egg hatching and larval release behavior. Carboxyl terminal arginine peptides synchronize larval release. The peptides originate from trypsin-like activity in the hatching eggs. In response to the peptides the female casts larvae into the water column. Incubating ovigerous crabs in trypsin causes larval release behavior, egg detachment and premature hatching. Preincubation of trypsin with a trypsin inhibitor eliminates these effects. Nanomolar concentrations of trypsin inhibitors evoke only larval release behavior. Because both peptides and trypsin inhibitors evoke larval release behavior and because trypsin inhibitors bind to both the peptide receptor and the enzyme with high affinity, the receptor binding site and trypsin catalytic site must be very similar. All necessary conditions for receptor binding can be met by substitution of a basic amino acid for the active site serine in the trypsin catalytic site. This substitution eliminates catalytic activity, maintains the binding affinity for trypsin inhibitors and increases binding strength for peptides. Supported by NSF DCB-8701544.

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Glycinamide Displays Sodium-like Activity on the Hamster Tongue. HARRY WMS. HARPER (Duck Engineering Design, 500 E. 63d St., New York, N.Y. 10021)

The amide of glycine has received a patent as a substitute for table salt (Cornelius, Eberts, and Sternberg. US# 4,066,799). The hamster *chorda tympani* response to 0.5 M glycinamide has the same magnitude as the response to 0.1 M NaCl. The pK of glycinamide is 7.8, and the pH of a 0.5 M solution is 4.5, so the molecule is fully ionized in this solution. Amiloride at .01 mM inhibits responses to 0.1 M NaCl by 81% on average. This same level of amiloride inhibits responses to 0.5 M glycinamide by 54% on average. Increasing amiloride concentration to 0.1 mM does not increase glycinamide inhibition. The **Diffusion Potential Model** of salt taste transduction predicts the magnitude of the inhibited responses to these salts, based on the liquid junction potentials they develop in contact with the interstitial fluid of the taste bud in the junctional complex which joins the taste cells to form the floor of the taste pore. These potentials can be calculated from the activities and mobilities of the ions in the solutions. The theoretical value for the difference between the inhibited responses (that is, the slope of the potential-response function) agrees well with experiment. (The prediction is 0.30, and the observation is 0.27) However, the absolute values of the predicted responses are substantially greater than the observed responses. Glycinamide has not previously been identified as an ion permeant in sodium channels. These observations provide valuable information concerning the effective size of sodium channels in general, and taste receptor sodium channels in particular.

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The anterior tongue receptive field specifies sodium taste in the rat. M. NITABACH, G. SCHWARTZ, A. SPECTOR, H. GRILL (Dept. of Psychology, University of Pennsylvania, Philadelphia, PA 19104).

Previous results have suggested that the chorda tympani innervation of the anterior 2/3 of the rat tongue mediates the gustatory recognition of sodium ions in solution. The present study adapts a paradigm developed by Nachman (*J.C.P.P.*, 55: 1124-1129, 1962) to further test this hypothesis. Subjects were male Sprague-Dawley rats, which were on a 22 hr. daily water deprivation schedule. All rats received a 10 min. two-bottle preference test between 0.2M NH_4Cl and 0.4M NaCl. All rats were 22 hr. water deprived at the time of the preference test. Before the start of the preference test, each rat was forced to sample each presented solution twice. The rats were divided into 3 groups: bilateral chorda tympani sectioned (CTX), glossopharyngeal nerve sectioned (GPX), or intact (INT). Within each of these groups, half of the rats were treated the night before the preference test with furosemide; these rats were in a state of body sodium deficiency (dep) at the time of testing. The other half of each group was untreated, and was thus in a state of body sodium balance (rep) at the time of testing. Consistent with the results of Nachman, we found that the INT and GPX groups exhibited a vastly different 0.4M NaCl preference depending on the internal state of the rats; the INT-rep and GPX-rep rats preferred the 0.2M NH_4Cl, while the INT-dep and GPX-dep rats completely preferred the 0.4M NaCl. These preferences were significant within the first minute of the test, and were thus probably based on taste rather than on post-oral effects of the solutions. The difference in preference between the sodium replete and sodium deplete rats is explained by the fact that the INT and GPX rats can specifically *recognize* sodium on the basis of taste. In striking contrast, CTX rats did not change their NaCl preference depending on their state of body sodium balance; both the CTX-rep and CTX-dep groups preferred the 0.2M NH_4Cl. This suggests that the CTX rats were unable to *recognize* sodium on the basis of taste. This study is the first to show that a specific taste submodality, sodium taste, is dependent on a particular tongue receptive field, the anterior field innervated by the chorda tympani nerve.

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Organization of amino acid taste information on single facial taste neurons of the marine catfish *Arius felis*. W. MICHEL and J. CAPRIO. (Department of Zoology and Physiology, Louisiana State Univ., Baton Rouge).*

In a previous report (Michel and Caprio, AChemS X, 1988) data were presented supporting the existence of several distinct neuron types for amino acids in *Arius felis*. In the present report, those findings are extended and interpreted in light of concurrent studies identifying the receptor site types for the amino acid stimuli in the same species. The amino acids tested, L-alanine, glycine, D-alanine, L-proline, L-histidine and L-arginine, were the most effective for the respective fiber types. Cluster analysis of the 42 fully characterized neurons identified two large groups of neurons and four smaller groups. The 16 neurons in the first group all responded best to D-alanine. The 19 neurons in the second group responded approximately equally to L-alanine and glycine. A significantly lower maximal response frequency and narrower mean breadth of responsiveness measure for the D-alanine sensitive neurons support the distinct nature of neurons forming the two major clusters. The smaller clusters contain cells that responded "best" to L-histidine (n=1), L-proline (n=2), L-alanine (n=2) and L-alanine/L-proline (n=2), respectively. The breadth of responsiveness measure for the 42 neurons (0.6 ± 0.2, SD) indicate that they are not narrowly tuned. The large variance around the mean values for any cluster demonstrates that individual cells comprising a cluster do not have identical response characteristics for the less effective stimuli. This variable sideband response is presumed to be due to either a differential representation of amino acid receptor site types on taste cells and/or differential innervation patterns of individual facial taste neurons onto the taste cells. Results of this study indicate, however, that the facial taste system of *Arius felis* provides sufficient information to code for multiple amino acid tastes.

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Coding in Olfaction: A Model Derived From Multidimensional Scaling Analysis of Responses to Complex Mixtures in Lobsters. MARIE-NADIA GIRARDOT AND CHARLES D. DERBY (Georgia State University, Atlanta GA)

The spatial distribution of artificial mixtures of crab, mullet, oyster and shrimp derived by MDS is relatively similar for behavioral and receptor cell responses, suggesting that the code for chemicals resides in the receptor population response. Population responses may vary in terms of their absolute response magnitude (ARM) and/or the pattern of responses across the population of neurons (ANP). Using the results of MDS analysis of responses of 30 olfactory cells to extracts of crab, mullet, oyster and shrimp at 0.005, 0.05 and 0.5 mM, evidence will be provided that stimulus concentration and stimulus quality are discriminated by separate population codes: stimulus concentration is not encoded by the ANP but is most likely encoded by the ARM, and stimulus quality is not encoded by the ARM but is most likely encoded by the ANP. Evidence will also be provided that "best" responders are neither more sufficient nor more necessary than "least" or "neither best nor least" responders for neural discrimination at the receptor level, but that all types of cells, whether high or low responders, are equally important in coding the quality of chemicals.

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Monoclonal Antibodies Specific for Cultured Olfactory Basal Cells. S. K. PIXLEY and L. LEMING (Dept. of Anatomy & Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267).

Olfactory receptor neurons are continuously generated in adult mammals, supposedly from basal cells in the olfactory epithelium. In order to investigate olfactory neurogenesis and interrelationships between different olfactory cell types, we have generated dissociated cell cultures from newborn rat pup nasal tissues which contain olfactory epithelia. Many cell types are present in these cultures. Olfactory basal cells and neurons have been identified by cell-type-specific antibodies. However, identification of other cell types is hampered because of the lack of sufficient cell-type-specific antibodies. Further antibody development is also needed because there are no satisfactory cell-surface binding, cell-type-specific antibodies which can be used to manipulate cultured cells. To develop such reagents, and cell-type markers, we have generated monoclonal antibodies specific for subsets of cells in dissociated nasal cell cultures.

Monoclonal antibodies were developed using antigen injections and in vitro presentation of antigen to mouse spleen cells. CB6F1/J mice were intraperitoneally injected with lightly fixed, then, (2 weeks later) live newborn Sprague-Dawley rat nasal tissue cells cultured for four days in vitro. Two weeks later, spleens from these mice were removed, dissociated into single cells and plated onto one day in vitro nasal cell cultures. Spleen cells were removed after a three day incubation, fused with X-63-Ag8.653 myeloma cells and plated onto peritoneal exudate cell feeder plates in HAT selective medium. Supernatants from fused cells were screened for presence of surface-binding antibodies specific for subsets of 2-4 day cultured nasal cells, using immunoperoxidase staining on lightly fixed cells. Positive supernatants were also screened using olfactory tissue sections. Cells from positive wells were dilution cloned. Out of many cell subset-specific clones, several produced antibodies specific for the cell surfaces of olfactory basal cells. No binding of these antibodies to other cultured cell types was observed. Further antibody characterization will include in vitro studies and Western blot analyses. These antibodies will be used to remove and purify olfactory basal cells from dissociated cell mixtures, or to specifically kill basal cells, allowing study of other cell types. The development of monolayer cultures with one or few cell types will be of great value in determining the lineage relationships between olfactory cells, and in studying olfactory receptor neurogenesis.

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Immunocytochemical investigation of olfactory neurons and synapses at the light and electron microscopic levels.

EDWARD W. JOHNSON, Ph.D., PAMELA M. ELLER, BRUCE W. JAFEK, M.D. (University of Colorado Health Sciences Center)

At the light microscopic level, olfactory neurons can be identified with a polyclonal antibody, designated anti-OMP, which recognizes a protein called olfactory marker protein (OMP), which has been isolated in the olfactory neurons of rats. Studies utilizing this antibody have shown its specificity for olfactory neurons, and possibly microvillar cells; both these cell types are found in the olfactory epithelium. OMP can also be localized in the olfactory bulb glomeruli, which are the sites of termination of olfactory neurons. An immunocytochemical procedure that involves anti-OMP as the primary label with subsequent peroxidase labeling (using an avidin-biotin protocol) has been used. A final step that results in deposits of a chromogen (DAB) at the immunoreactive sites allows for visualization of cells that contain OMP. After an intra-aortic perfusion of rats with a fixative that contains paraformaldehyde, picric acid and 0.2% glutaraldehyde, the immunocytochemical procedure allowed us to specifically label olfactory neuron dendrites (that extend to the mucosal surface), somata and the fila olfactoria (olfactory neuron axons that project to the olfactory bulb glomeruli). Olfactory bulb glomeruli were also labeled. Labeling of microvillar cells with anti-OMP will also be investigated. Furthermore, this immunocytochemical technique can identify anti-OMP labeled cells, processes and terminals at the electron microscopic level after the DAB is osmicated. Ultrastructural studies are continuing with the goal of identifying anti-OMP immunocytochemically labeled cells and processes in the olfactory epithelium and anti-OMP labeled terminals in the glomeruli of the olfactory bulb.

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Immunocytochemistry in *Necturus* taste buds: Is GABA a neurotransmitter in taste cells? SHASHI B. JAIN and STEPHEN D. ROPER (Department of Anatomy & Neurobiology, Colorado State University, Ft. Collins, CO 80523 and the Rocky Mt. Taste & Smell Center, Denver, CO 80262)

Chemical synapses have recently been observed between cells in the taste bud and between cells and afferent nerve fibers in *Necturus* (Delay & Roper, 1988, J. Comp. Neurol. 277, 268). The neurotransmitter(s) released at these synapses are not known, although it has been speculated that synapses are monoaminergic or cholinergic. We report here that GABA-like (henceforth, GABA) immunoreactivity, studied at the light microscopic level, has also been localized to taste cells and sensory fibers innervating taste buds in *Necturus*. Lingual epithelium was dissected from the animal and fixed in formaldehyde-glutaraldehyde. Thick (20 microns) sections were cut with a cryostat and treated with primary anti-GABA antibody (rabbit) obtained from Chemicon. Control tissues were treated with rabbit serum (anti-GABA free). Secondary antibody and biotinylated peroxidase (ABC kits) were obtained from Vector Laboratories and Pel-Freez. Control tissues showed no or weak backgound staining in the lingual epithelium. The extent of background staining increased with increasing concentration of glutaralydehyde in the fixative (range=0% to 2%). Tissues treated with anti-GABA antibody showed strong immuno-reactivity at the base of the taste bud and in the nerve fibers innervating the taste buds. Some taste cells also stained with anti-GABA immuno-reactivity. These findings suggest that GABA may be a neurotransmitter in taste buds in *Necturus*. Experiments to confirm this finding at the electron microscopic level and to test for other amino acid neurotransmitters are in progress.

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Taste Buds of the Foliate and Fungiform Papilla Compared for Two Strains of Mice. ROBIN F. KRIMM and INGLIS J. MILLER, JR. (The Bowman Gray School of Medicine, Wake Forest University)

It was reported recently (Miller and Whitney, Neuro. Abs. 13:1049) that SWR/J mice, which are tasters (with lower thresholds) for sucrose octaacetate, have more vallate taste buds than a non-taster (C57BL/6J mice) strain. Taste buds were counted for the foliate papilla and the fungiform papilla in C57BL/6J and SWR/J mice. Each mouse was sacrificed by an overdose of ether, decapitated, and the head was placed in Bouins fixative. After fixation, the tongue was removed, and the foliate papillae were embedded in paraffin. Serial sections were cut, mounted on slides and stained with hematoxylin and eosin. The sections were examined in order to count taste buds, and taste buds were scored in the section in which a taste pore was identified. In order to count fungiform taste pores, tongues were stained with Methylene blue or Ponceau S and taste pores on the surface were counted with the aid of videomicroscopy. Taste bud counts using this procedure were verified for a small piece of tissue from each tongue using the light microscopy procedure described above. Preliminary results indicate that SWR/J mice have slightly more taste buds in the foliate papillae (mean = 66.8, range 62-84) than C57BL/6J mice (mean = 61.3, range 50-74).

Supported by NIH Grant NS 20101.

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Human Fungiform Papillae and Taste Pore Density. FRANK E. REEDY, JR. AND INGLIS J. MILLER, JR. (The Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

We count taste pores in living human subjects in order to study the relationship between taste bud distribution and taste perception. Fungiform papillae and taste pores were mapped and counted on the tongue tips in 8 living, human subjects using videomicroscopy. The subjects were all university personnel comprised of 6 males and 2 females, ranging in age from 17- 33 years. Total fungiform papilla density ranged from 22.6 to 73.6 papillae/cm^2 with a mean of 46.4 $\pm$ 17.8 pap/cm^2 (s.d., N=8). Taste pores were quantified in 5 subjects with a mean fungiform papilla density of 42.7 pap./cm^2. An average of 43 pap/subject was studied for taste pores, and a mean of 39 pap/ subj. (91%) contained taste pores. The papillae were contained in an average area of 0.85 cm^2 of the tongue tip with equivalent densities on both sides of the midline. The number of taste pores/papilla ranged from 0-22 among subjects with a mean of 4.82 taste pores/gustatory papilla and 4.39 taste pores/total fungiform papillae (including those w/o pores). The density of taste pores in the sampled region of the tongue tip ranged from 97 to 511 taste pores/ cm^2 with a mean of 269 taste pore/cm^2. These observations correspond only to the upper range of values from human cadaver tongues. The disparities between living subjects and cadaver tongues could be attributed to different methods and different subject populations. Supported by NIH Grant NS 20101 from NINCDS.

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A Rapid Method of Collecting Taste Tissue from Rats and Mice. ANDREW I. SPIELMAN, JOSEPH G. BRAND and LINDA WYSOCKI (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104).

Taste papillae removed by surgical excision from rats and mice contain surrounding epithelial and muscle tissue far in excess of taste buds. It often involves tedious and lengthy procedures. The purpose of this study was to develop a rapid technique to harvest taste papillae from rats and mice with minimal contamination. Vallate, foliate and fungiform papillae from rats and mice were punched out with the aid of glass capillary tubes. Depending on the species and papillae, the internal diameter of the glass capillaries were varied from 0.022-0.039 inches (0.55-1 mm) to fit the perimeter of papillae and include the least amount of epithelium. This was demonstrated by histological sections and SEM of the collected specimens. Removal of papillae was possible at a rate of 8 per minute. This method should improve collection of tissue enriched in taste cells especially when the aim of collection is a rapidly degrading cellular component such as mRNA.

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Cell lineage in the mudpuppy, *Necturus maculosus*

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Taste cells of the mudpuppy, *Necturus maculosus*, are unusually large and are amenable to a variety of physiological tests. Three cell types have been identified in *Necturus*: Merkel-like basal cells, dark cells and light cells (Farbman and Yonkers, '71; Delay and Roper, '88). Although the cell lineage of taste cells has been examined in mammals, showing that basal cells differentiate into dark cells and dark cells into light cells, the interrelationship of cell types in amphibian taste buds is unclear. In order to define this relationship we have undertaken a tritiated (^3H) thymidine study to follow one generation of taste cells throughout their lifespan. Adult *Necturus* were kept in aquaria at room temperature. Each mudpuppy was given two injections of ^3H thymidine (each injection: 1.5μC/gm body weight) 12 hours apart. After injection, animals were sacrificed at various intervals up to 40 days. Following standard procedures, taste buds were examined using autoradiographic light microscopy and then selected sections were reembedded and sectioned for electron microscopy. Tritiated-labelled taste cells were observed within the taste buds one day following injection. These labeled cells were located towards the side and base of the buds and did not extend processes to the taste pore. We tentatively identify these cells as undifferentiated basal cells. Labelled nuclei appear in all cell types during the 40-day time span. At the later time intervals, a preponderance of labelled light cells were identified. In one case, a labelled senescencing cell was observed being extruded from the taste pore.

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Ouabain-sensitive, potassium-dependent ATPase activity in the mouse taste bud and neighboring lingual epithelium. MICHIO MORITA and STEPHEN D. ROPER (Department of Anatomy & Neurobiology, Colorado State University, Ft. Collins, CO 80523 and the Rocky Mt. Taste & Smell Center, Denver, CO 80262)

Investigations by DeSimone and others indicate that active transepithelial ion transport, especially of sodium, is intimately coupled with taste transduction in some, but not all animals. An essential feature of this schema is that passive influx of Na through the apical membrane of taste cells is followed by active sodium pumping across the basolateral membrane. Although there is evidence for amiloride-sensitive, passive Na influx in taste cells from some species, the presence of basolateral sodium pumps has not yet been demonstrated. We present electron microscopic cytochemical evidence here for the existence of sodium pumps in taste cells. Ouabain-sensitive, potassium-dependent ATPase activity (i.e. the sodium pump) was examined in the mouse taste bud and neighboring lingual epithelium by the modified techniques of Mayahara, et al (1980). In the taste bud, lead (Pb) deposits (the final cytochemical reaction product at the site of ATPase enzyme complexes) appeared on the basolateral membrane of light and dark taste cells. However, relatively few Pb deposits were detected on the membrane of dark cells. No Pb deposits were seen on the membrane of microvilli or apical protions of cells in the taste pore. In the neighboring lingual epithelium, Pb deposits appeared on the basolateral membrane of flattened keratinocytes, whereas they were detected on the entire plasma membrane of cuboidal cells located in the stratum basale. These findings suggest that Na pumps (the sites of ATPase complex) exist on the basolateral plasma membrane of the taste cells as well as neighboring lingual epithelial cells. However, the presence of reaction product on taste cells as well as non-taste epithelial cells is not consistent with transepithelial transport occuring exclusively through taste buds. Experiments are underway to quantify the distribution of Na/K dependent ATPase on taste and non-taste epithelial cells to resolve this latter point.

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Innervation of Extra-Oral Taste Buds by the Superior Laryngeal Nerve. MICHAEL WHITCOMB, KENT NICKLAS & SUSAN TRAVERS (College of Dentistry, The Ohio State Univ., Columbus, OH 43210)

The purpose of the present study was to quantify and describe the distribution of taste buds in the rat pharynx and larynx and determine their somatotopic innervation by the superior laryngeal nerve (SLN). The extra-oral distribution of taste buds was examined in 10 intact rats, and in 4 rats in which the SLN had been interrupted bilaterally, by removing a 1-3mm section, 14 days prior to sacrifice (in 3 rats, the central end of the nerve stump was also soaked in formo cresol to devitalize the remaining portion of the nerve). The pharynx and larynx were dissected from formalin-fixed rats, decalcified, embedded in paraffin, sectioned (10u), and stained with routine hematoxylin and eosin. Taste buds were counted by drawing serial sections and plotting the buds relative to anatomical structures. Adjacent sections were compared to prevent double counting of buds. In the 10 normal animals there was a mean total of 140.4 +/- 25.0 extra-oral taste buds. Thirty-two of these buds were on the nasopharynx (NP). The remaining buds were located on the palatopharyngeal eminence (PPE) (n=5.6) or were associated with the laryngeal cartilages (n=102.8) in a continuous distribution extending from the epiglottis to the vocal folds. The majority of the laryngeal taste buds were located on or caudal to the aryepiglottic folds. Bilateral section of the SLN resulted in a large reduction in the number of extra-oral taste buds that could be identified (n=36.8 +/- 31.8, $p<.001$). Most of the remaining buds were located on the NP (n=26) or PPE (n=6). The number of buds associated with these structures were comparable to the number observed in intact rats. These results suggest that, although there are many taste buds on the rat epiglottis, the majority of extra-oral taste buds in this species are located further caudally. The SLN appears to innervate virtually all the laryngeal taste buds, but not the buds associated with the NP or PPE. Work in progress is investigating the role of the IXth nerve and the pharyngeal branch of the Xth nerve in innervating the remaining extra-oral taste buds.

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Time Course of Reinnervation of the Olfactory Bulb After Transection of the Primary Olfactory Nerve in the Pigeon. R. A. JENNINGS, J. C. WALKER, AND J. H. REYNOLDS (Bowman Gray Technical Center, Research and Development, R.J. Reynolds Tobacco Co., Winston-Salem, N.C. 27102)

Horseradish peroxidase (HRP) histochemistry was used to study the time course of reinnervation of the pigeon olfactory bulb following simple transection of the primary olfactory nerve. At selected time intervals (1 to 32 days) following transection of the right olfactory nerve, both nasal cavities were irrigated with a concentrated solution of HRP. Following intracardiac perfusion, four days after nasal irrigation, 40 micron sections of olfactory bulb were processed with tetramethylbenzidine and hydrogen peroxide to visualize the olfactory receptor axon terminals in the olfactory nerve and glomerular layers of the bulb. A Zeiss IBAS 2000 image analysis system was used to quantitatively compare reinnervation of the bulb on the transected side to the innervation of the left control bulb. With an interval of 8 days between transection and HRP nasal irrigation, approximately 12% of the bulb was innervated relative to the control bulb. Reinnervation at that time point was largely confined to the medial regions of the bulb. Thereafter, a consistent increase in area of reinnervation was observed over time. Reinnervation appeared to be nearly complete at 32 days when a continuous band of reaction product, quite similar to that seen in sections from the control bulb, was observed. These results complement work by Kiyohara and Tucker (1978) who found that the reconstituted olfactory nerve of the pigeon exhibited normal electrophysiological responses to odorants as early as 30 days after transection. The present results may also be used to guide odor psychophysical comparisons of normal and newly reconstituted peripheral olfactory systems.

Kiyohara, S. and Tucker, D. 1978. Activity of new receptors after transection of the primary olfactory nerve in pigeons. *Physiol. Behav.* 21: 987-994.

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Innervation of the Vomeronasal Organ in Rodents by peptidergic nerve fibers immunoreactive for Substance P and Calcitonin Gene-Related Peptide (CGRP). THOMAS E. FINGER, MARY WOMBLE (Univ. Colorado School of Medicine & Rocky Mountain Taste & Smell Center, Denver CO 80262), & WAYNE L. SILVER (Dept. Biology, Wake Forest Univ., Winston-Salem, NC 27109).

The vomeronasal organ, a specialized sensory organ in the nasal cavity, provides input to the accessory olfactory system. In rodents, stimulus access to this organ is controlled largely by a vascular pumping system which expands and constricts the passageways within the vomeronasal organ. The sensory epithelium of the vomeronasal organ lines one side of the cavity; respiratory-type epithelium lines the other side. In our studies of the peptidergic innervation of the nasal cavity, we noted that the non-sensory surface of the vomeronasal organ has a comparatively dense peptidergic innervation, as visualized by antisera directed against CGRP or substance P. Cryostat sections taken from the vomeronasal organ of either rats, mice or chinese hamsters showed similar patterns of immunoreactivity. Numerous peptidergic fibers occurred in the non-sensory epithelium of the organ as well as in the epithelium of the underlying cavernous tissues. Double label studies utilizing primary antisera raised in different species indicate that the immunoreactivities to the two peptides co-localize within virtually all of the peptidergic fibers. Only rare peptidergic fibers occur within the vomeronasal sensory epithelium; this density is approximately the same as that in the olfactory epithelium. Since the peptidergic fibers are associated with the nonsensory epithelium, we hypothesize that the peptidergic fibers do not play a direct role in vomeronasal sensation. Rather, the fibers' association with the cavernous tissues coupled with the fact that peripheral release of these bioactive peptides alters local blood-flow in tissues, leads us to speculate that the peptidergic fibers play a role in the vomeronasal pumping mechanism. The peptidergic fibers may be active during normal pumping, or may be activated only during protective reflexes.

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Neonatal Development of the Vomeronasal and Olfactory Systems in the Gray Opossum, Monodelphis domestica. RUU-TONG WANG and TRACY L. SOLTESZ, (Department of Anatomy, Marshall University School of Medicine, Huntington, WV 25704) and MIMI HALPERN (Department of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, NY 11203.)

Adult gray opossums possess clearly differentiated vomeronasal (VN) and olfactory organs (Wang and Halpern, 1988). Neonatal opossums are born at a developmental stage comparable to embryonic stages in other mammals and thus are good subjects for studying the development of their nasal chemosensory structures. We have investigated the development of VN and main olfactory organs and the main and accessory olfactory bulbs (MOB and AOB) in eight groups of neonate opossums aged 0, 3, 5, 7, 14, 21, 25 and 28 days (n= 3 to 5 in each group). At birth, a pair of primordial VN organs can be differentiated from the olfactory epithelium located antero-medially along the wall of the nasal septum. Each organ contains a population of simple epithelial cells mixed with some mesenchymal tissues and hemopoietic elements. A VN lumen opens into both nasal and oral cavities. Mitoses are seen throughout the VN and olfactory organs and the primordial MOB of the new born opossums. The AOB can not be distinguished at this stage. Subsequently, with increasing epithelial thickness, mitoses in the VN and olfactory sensory epithelia are restricted to the supporting cell layer and basal cell layer. In 3-day-old neonates, very few olfactory glomeruli are present in the MOB. The differentiation of the AOB, with glomeruli, mitral cells and inner granule cells, is discernable at 14 days of age. Although the VN gland differentiates from the VN organ by 7-days, the formation of secretory acini is not clear until 28 days of age. This study suggests that neonatal maturation of the VN system in the gray opossum occurs later than that of the olfactory system. Furthermore, in both nasal chemosensory organs, the commitment of stem cells for genesis of supporting cells or sensory neurons occurs early in neonatal development. (Supported by NIH grants RR 05870 and NS 11713).

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Effects of Methyl Bromide on Rat Olfactory System. L. HASTINGS, M. MILLER, D. MINNEMA AND J. EVANS (Dept. of Environmental Health, Univ. of Cincinnati).

Methyl bromide (MB), widely used as a fumigant, is highly toxic, producing lesions in a number of tissues. A primary toxic response to inhaled MB occurs in olfactory epithelium while the respiratory epithelium is largely spared. The specific site of damage appears to be in the olfactory sustentacular cell population. To further study the nature and extent of damage produced by MB exposure and to examine recovery of function after exposure, a multifacit approach was used which includes behavioral, morphological and neurochemical endpoints. Thirty adult Long-Evans rats were exposed to 200 ppm MB for 4 hrs per day, 4 days a week for two weeks. Fifteen control rats were exposed to filtered air only. Prior to MB exposure the rats were trained on a buried food pellet test. In this test, rats are food deprived and then trained to find a food pellet buried beneath the bedding in the cage. Exposure to MB for 4 hours greatly impaired recovery of the buried food, indicating olfactory impairment. However, even with continuous exposure, recovery occurred until by day 4 of exposure, olfactory function was essentially normal. Extensive damage to the olfactory epithelium was evident after day 1 of exposure; repair of the epithelium was in progress by day 4. The third parameter, level of carnosine in olfactory bulbs (as in index of olfactory epithelium damage) is now in progress. In conclusion, the olfactory epithelium appears to be a resiliant tissue, capable of repair and recovery of function, even when exposed chronically to a toxic agent.

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Post-Viral Olfactory Dysfunction

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Fourteen patients suffering from post-viral olfactory dysfunction have been studied at the Rocky Mountain Taste and Smell Center, Denver, Colorado. Each patient completed the Connecticut Chemosensory Research Center (CCCRC) questionnaire. Chemosensory testing consisted of an n-butyl alcohol threshold test, a 7-item odor identification test, the University of Pennsylvania Smell Identification Test (UPSIT), a Q-tip spatial taste test, sucrose threshold tests, a citric acid magnitude estimation and electrogustometry. Olfactory biopsies were obtained and processed for electron microscopic evaluation. Ultrastructural examination of the biopsy specimens demonstrated differences between patients who were classified hyposmic based on their chemosensory test results and those who were classified anosmic. Results of the chemosensory testing and the olfactory biopsy results will be presented in detail.

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Logarithmic Transformation Of Magnitude Estimation Data HARRY LAWLESS (Cornell University).

Data from magnitude estimation procedures are sometimes submitted to logarithmic transformation before analysis. Reasons for transformation have included 1) assumptions about an underlying psychophysical power law, 2) inter-subject "normalization" is unneccessary when geometric means are taken (often via logs) in non-modulus procedures, and 3) the need to normalize lognormal distributions, in order to better approximate the assumptions of statistical testing and thus have accurate estimates of the probability of Type I error. Logarithmic transformation has sometimes been avoided in the chemical senses, mainly due to the problem of "zero" judgments in the data. In consumer testing, the question arises as to whether log transformation can enhance statistical differentiation among products. Data from magnitude estimation, category ratings and line scales were gathered from consumer tests using six sensory continua. F-ratios for product differences were examined as indices of the sensitivity of scaling procedures. As expected, F-ratios tended to increase after log transformation of magnitude estimation data, and tended to decrease after log transformation of category scale and line scale data. Improvements in F-ratios were associated with reduced skew, and better approximation of normal distributions after transformation. However, although log transformation tended to lessen the deviations from normality for magnitude estimation data, the majority of distributions were still significantly non-normal after transformation. Statistical improvement after log transformation was less evident in a group of students than in a group of homemakers/consumers. Students tended to generate higher F-ratios with magnitude estimation and had a lower degree of positive skew in their untransformed raw data. The statistical benefit of log transformation may lie in reducing the impact of outlying data from extreme subjects and reducing mean square error in subject-by-product interaction terms.

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Comparison of Canonicial Variate and Principal Component Analyses of Sensory Descriptive Data. A. C. NOBLE (University of California, Davis) H. HEYMANN (University of Missouri, Columbia)

Descriptive analysis ratings for Cabernet Sauvignon wines from 4 regions and Chardonnay wines from three vintages were evaluated by principal component analysis (PCA) and canonical variate analysis (CVA) using individual wines or group classes (region or vintage) as the classification variables. PCA and CVA-Wine analyses provided similar results for both data sets, permitting the examination of the relationship of the sensory variables and visualization of patterns in the data. In contrast, the CVA-Class analyses emphasized the significant differences among regions or vintages rather than reflecting the differences among the wines overall. For example, CVA-Class (region) for the Cabernet Sauvignon wine separated wines from the cool Southern region from the rest on the basis of bell pepper character alone. Although green bean aroma and vegetative flavor by mouth were scored highest in five of the six Southern wines, they varied extensively within regions and were not weighted heavily on the first CVA-Class axis, which separated regions. The major advantage of CVA is the ability to determine significant differences between wines or classes, which PCA cannot do. However, CA-Wine and PCA analyses facilitate understanding the structure of the data by illustrating the overall relationship among the variables and the samples better than do the CVA-Class analyses.

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Hunger Level Affects Hedonic Differences between Tongue Dip and Swallow. ROBERT J. HYDE (San Jose State University, San Jose, CA)

Several commercial sweet foods have been judged to be sweeter and more liked during swallowing compared to tongue dipping (Hyde, 1987). "Loci Difference Analysis" has revealed that compared to more palatable fully-carbonated Pepsi, flat Pepsi evoked a markedly diminished magnitude of difference between judgments for tongue dip (TD) compared to swallowing (S) for sweetness and degree of liking (Hyde, 1988). Presently, TD and S responses from 44 college women (normal weight) were evaluated to test the hypothesis that diminishing hedonic tone for repeatedly ingested chocolate milk (sensory-specific satiety) would be associated with loss of loci differences in hedonic tone. On 100 mm visual analog scales, Ss rated initial hunger level and sweetness and degree of liking, first while dipping the tongue into commercial chocolate milk (room temperature) and then while swallowing the beverage. Ss gave sweetness-hedonic judgments for three10-ml chocolate milk samples in succession, ingested six samples with 30-sec intervals inbetween, and then judged a final three samples before giving another hunger rating. To obtain groups of Ss more homogeneous in their sensory responses and physiologic state, Ss retained for analysis were those whose mean sweetness judgments during S for the first three samples exceeded those for TD by at least 10 mm on the analog scale. "Hungry" Ss (n = 12) were those whose hunger ratings initially exceeded 40 mm and fell by at least 25 mm after all samples were ingested. "Sated" Ss (n = 13) were those whose hunger levels remained low, within ±10 mm, throughout the experiment. The pattern for the following results resembled that for the initial 44 Ss. From start to finish, both groups of Ss had mean judgments for sweetness during S that significantly exceeded those obtained during TD ($p<0.001$). Compared to sated Ss after repeated ingestion of chocolate milk, hungry Ss showed a larger fall in mean hedonic responses and a loss of loci differences in degree of liking for S compared to TD ($p<0.001$). Resistance of reward neurons to habituation for chocolate milk during satiety would explain the reduced magnitude of sensory-specific satiety in the nonhungry Ss herein. People may find sweet foods pleasurable for longer periods when they are full compared to when hungry.

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Ambient Odor and Shopping Behavior
SUSAN C. KNASKO (Monell Chemical Senses Center)

Shopping behavior was studied for 18 days in a store which was experimentally fragranced on an every-other-day basis. Two different scents were used in two separate sections of the store. Daily sales figures for the entire store and the two scented areas were obtained. The number of customers who walked into the two scented departments and the amount of time they spent there was determined from surveillance videos which recorded daily for two hour periods.

The author would like to thank the Firmenich Company for supplying the odorants and odor delivery system and for funding the rental of the video equipment.

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Time-Intensity Studies of Citrus Bitterness Compounds: Influence of Threshold Level R. L. ROUSEFF (University of Florida), WILLIAM E. LEE III (University of South Florida), & C. A. HUEFNER (University of Florida).

Time-intensity (T-I) techniques were used to study the behavior of the compounds naringin and limonin, the two major bitter compounds found in citrus products. These compounds were isolated from citrus and presented to screened panelists in a series of aqueous solutions. Individual bitterness taste thresholds were established using caffeine. Using T-I techniques, it was established that maximum bitterness was perceived within a few seconds. This maximum was then followed by a gradual decline in perceived bitterness intensity. Each compound produced a characteristic response. Total bitterness time was greater from limonin solutions than from equibitter naringin solutions. Subjects with high thresholds tended to display a response curve that had a slower time to attain maximum intensity, lower overall intensities, and more rapid disappearance of the perception. Low threshold subjects had responses that showed higher overall intensities and significantly longer persistence of the bitterness. Such results may help to explain why some people have a very strong reaction to bitterness in citrus compounds.

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Sensory Evaluation of Oral Products by Tasters and Non-tasters of PTC. JOHN LABOWS, DAVID INGERSOLL, ROBERT CAGAN, RHODA HARRISON and CAROLYN WINTER (Corporate Technology Center, Colgate-Palmolive Co., Piscataway, NJ 08854)

The sensitivity of certain segments of the population to the bitterness of phenylthiocarbamide [PTC] has been known for over 50 years and has been used to chart genetic patterns in human populations. This sensitivity has been correlated to the perception of bitter in common food materials like saccharin and caffeine and to the bitterness of potassium. However to our knowledge, this difference in bitter sensitivity has not been exploited in the evaluation of oral products. Typical dentifrice and mouthrinse formulations contain bitter tasting components, such as saccharin and sodium lauryl sulfate, while tartar control formulations contain potassium. A panel of PTC tasters and non-tasters of PTC was formed to evaluate the effectiveness of certain flavors in masking the bitterness of experimental tartar control dentifrice and mouthrinse formulations. Magnitude estimation studies determined the intensity ratings of bitterness and six other sensory attributes. If no differences were found between tasters and nontasters for relatively low mean levels of bitterness for a given formulation, the flavor was effective in masking bitter. Differences in assessments is an indication of ineffective masking. Associations of bitter sensitivity with other attributes were also found. This approach makes use of a specific population, sensitive to a negative taste attribute, to judge flavor acceptability.

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Taste Test Hedonic Ratings, Post-Meal Hedonic Ratings and Consumption as Measures of Preference for a Salted Entree.
RICHARD POPPER (U.S. Army Natick RD&E Center)
OWEN MALLER (U.S. Army Natick RD&E Center)
ARMAND V. CARDELLO (U.S. Army Natick RD&E Center)

Fifty-seven subjects provided sensory and hedonic ratings of an entree prepared with four different levels of added salt. All subjects participated in a taste test in which they were served small samples of each entree. Subjects also participated in one of two meal conditions, in which they were served a 460 g portion of the entree at lunch, either by itself or accompanied by soup, starch and dessert. Meal tests were conducted once a week over a period of four weeks; each week subjects were served the entree with a different level of added salt (the salt levels of the other items in the meal were held constant). Sensory and hedonic ratings were collected at the end of the meal, and the amount consumed at the meal was determined by measuring plate waste. There were no significant differences in saltiness or hedonic ratings between the two meal conditions. Although there were differences between the taste test and meal ratings, the most preferred level of added salt was similar in the two conditions. On the other hand, the amount of the entree consumed did not correlate highly with hedonic ratings. In addition, the optimal level of added salt, determined by the amount consumed, differed in some cases from the optimal level indicated by hedonic ratings. This finding agrees with results previously reported by Lucas and Bellisle (1987).

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Dendritic Proteins of the Sex-Pheromone Specific Sensory Neurons of Moths.
RICHARD G. VOGT and MICHAEL R. LERNER (Section of Molecular Neurobiology, Yale University School of Medicine).

The olfactory sensilla of moths are well known for their remarkable sensitivity and specificity to sex-pheromone. We are studying the receptor/transductory mechanisms of pheromone detection by characterizing the protein biochemistry of the sensory dendrites. In previous studies (Vogt *et al.*, 1988) a radiolabeled photoaffinity analog of the *Antheraea polyphemus* (silk moth) pheromone was shown to covalently attach to a 69 kDa membrane protein of the *A. polyphemus* sensory dendrites. This protein appeared to be uniquely associated with this membrane, and natural pheromone was shown to displace analog binding in a selective manner. These properties are consistent with this 69 kDa protein being a pheromone receptor protein. Now, we have injected ^{35}S-methionine into developing adult moths, including *A. polyphemus* and *Lymantria dispar* (gypsy moth). Sensilla were isolated from animals at adult emergence. The isolated sensilla contain only cuticle, the sensilla lumenal fluid with its extracellular pheromone binding proteins and enzymes, and the sensory dendrites. This preparation contains no detectable cellular material from other sources. The isolated sensilla were sonicated to dissociate these components, and then centrifuged (100,000 x *g*) in order to separate the membrane (pellet) from the soluble (supernatant) components. The membrane preparation was analyzed by SDS PAGE and fluorography. The major protein of the *A. polyphemus* membrane appeared to be a cluster at 69 kDa as well as tubulin and actin. The major proteins of the *L. dispar* membrane was also a cluster at 69 kDa as well as a cluster around 28 kDa and tubulin. The 69 kDa proteins of both species and the 28 kDa group of *L. dispar* were only observed in the olfactory membrane. Tubulin and actin were also the major proteins labeled in similar preparation of thoracic ganglia. The abundance and the apparent uniqueness of the 69 kDa and 28 kDa proteins suggests that they play a significant role in the olfactory process.

Vogt, R.G., Prestwich, G.D. & Riddiford, L.M. (1988) *Proc. Natl. Acad. Sci., USA* 263, 3952-3959.

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Molecular cloning of olfactory-specific cytochrome P450 and UDP glucuronosyl tranferase: candidate signal termination and odorant clearance enzymes. D. LAZARD, K. ZUPKO, J. HELDMAN, P. NEF AND D. LANCET (Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel).

Olfactory-specific signal transduction molecules identified to date include the G_s-protein, adenylate cyclase, cAMP channels and odorant carrier proteins. We describe here the identification and molecular cloning of two new olfactory-specific molecules that possibly take part in chemosensory signal processing. These are rat olfactory cytochrome P450 (P450olf1), and bovine olfactory UDP glucuronosyl transferase (UDPGT). Counterparts of these enzymes in liver and other tissues, respectively catalyse stage I and II drug detoxification, i.e. hydroxylation and glucuronidation. They also play a role in the metabolic modification of steroids, prostaglandins and lipids. P450olf1 and olfactory UDPGT are expressed uniquely in olfactory mucosa. Both are membrane-anchored, likely to be present in endoplasmic reticulum of one or more cell types of the chemosensory tissue. P450olf1 was cloned by screening a rat olfactory epithelial λ-gt11 cDNA library with a probe derived from a liver phenobarbital-induced P450b enzyme. Olfactory UDPGT was cloned from a similar bovine library using oligonucleotide probes, based on partial amino acid sequence of the major olfactory-specific glycoprotein gp56 (Kropf et al. Neurosci. Abst. **13** :1410, 1987). The cloned enzymes may be related to results of previous reports, showing that olfactory epithelium contains high activity of enzymes that metabolize drugs and xenobiotic chemicals, including odorants. By analogy to their role in other cells, and based on the observed tissue specificity, we propose that the two newly identified enzymes are important in odorant processing, including olfactory signal termination and odorant clearance. Intriguingly, they could also affect olfactory response spectra, by converting weak odorants into potent ones, or *vice versa*. As proposed previously, such olfactory enzymes could constitute multigene families, like the hypothetical odorant receptors, so as to cope with the diversity of odorants.

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Intermetabolic Relationships among Phospholipids in Catfish Taste Epithelium. JOSEPH G. BRAND, JOSEPH L. RABINOWITZ and T. HUQUE (Monell Chemical Senses Center, 3500 Market Street and Veterans Administration Medical Center, Philadelphia, PA 19104, U.S.A.)

Taste transduction may involve several types of ionic and molecular events including modulation of ion channels, direct ligand gating of ion channels and production of one or more types of second messengers. Because the potent taste stimulus for the catfish, L-alanine, stimulated production of inositol triphosphate in taste epithelium, we determined the metabolic fate of phosphate (^{32}P), ^{14}C(acyl)-phospatidyl choline (PC) and ^{14}C (acyl)- lyso-phosphatidylcholine (LPC) in tissue slices of catfish (I. punctatus) taste epithelium. When [^{32}P] sodium phosphate was incubated with epithelium, all phospholipids assayed became labeled. Maximal incorporation occurred near 20 mins for lysophosphatidylcholines (LPC), phosphatidylcholines (PC) and phosphatidylinositols (PI). The phosphatidylethanolamines (PE) and phosphatidylserines (PS) labeled more slowly. The label in LPC and PC declined from 20 min to 120 min, while that of the other fractions increased or was stable over the 120 min time period. Starting with [^{14}C acyl] PC in the media, label was found within minutes in all of the phospholipids assayed, with maximums occurring near 20 min. Label appeared in free fatty acids, mono- and diglycerides, triglycerides and methyl esters (AG) more rapidly (5 min) and declined rapidly as phospholipids became labeled. Within minutes of the addition of [^{14}C acyl] LPC, the ^{14}C of the acyl group was detected in the AG fraction and later in all the phospholipids. The PC fraction was maximally labeled by 40 mins. Label slowly disappeared from all fractions during the next 80 mins. These experiments indicate that there are active and rapid exchanges, degradations, syntheses and utilization of phospholipids in the taste organ of this animal.

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Stimulus Amino Acids Elicit Rapid Increase in Intracellular Calcium In Dissociated Olfactory Neurons. DIEGO RESTREPO (Monell Chemical Senses Center, Philadelphia, PA) and RICHARD C. BRUCH (Northwestern University, Evanston, IL).

Previous observations indicated that stimulus amino acids elicit rapid production of inositol trisphosphate (IP_3) in isolated olfactory cilia from the channel catfish (I. punctatus). The ability of IP_3 to mediate calcium release from intracellular stores and its role in stimulus-evoked increases in intracellular calcium (Ca_i) in olfactory neurons were therefore investigated. Isolated microsomes from the olfactory epithelium sequestered calcium in a Mg^{2+}/ATP-dependent manner. Addition of equimolar (5 uM) amounts of IP_3 or the calcium ionophore A23187 to preloaded microsomes evoked rapid release of 30% and 90%, respectively, of the sequestered calcium. Basal (20-100 nM) and amino acid-stimulated (5-10 fold increse) Ca_i was measured in dissociated olfactory neurons using the fluorescent Ca^{2+} indicator Fura-2. Morphologically identified olfactory neurons (95% viability with trypan blue) were obtained following treatment of the olfactory epithelium with papain in Ca^{2+}/Mg^{2+}-free buffer. These treatments did not affect L-[^{3}H]-alanine binding in isolated cilia. Conversion of the membrane-permeable acetoxymethyl ester of Fura-2 to the calcium-sensitive free acid in olfactory neurons was confirmed by spectral analysis and the calcium response of the converted dye. In Fura-2-loaded olfactory neurons, both L-alanine and potassium-induced depolarization elicited rapid increases in Ca_i. Taken together, these results suggest that IP_3-mediated release of calcium from intracellular stores contributes to stimulus-induced increases in Ca_i in olfactory neurons.

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Patch Clamp Recordings From Dissociated Rat Taste Cells. M. SCOTT HERNESS. (The Rockefeller University, New York, NY 10021)

A procedure was devised to acquire patch clamp recordings from dissociated taste cells of the rat tongue. Cells were dissociated from the rat tongue by blocking small sections containing either foliate, circumvallate, or anterior portions of the dissected tongue procured from an anesthetized rat. Tissue was incubated in a bicarbonate-buffered divalent-free Hank's solution containing the enzyme papain in a 5% CO_2 incubator at 37° Celsius. After approximately two hours, the tissue was placed in a serum-free L15 medium with glucose. The epithelium could be easily removed with fine dissecting forceps. Light mechanical agitation then caused epithelial cells, including taste cells, to dissociate themselves from the tissue. Taste cells could be easily identified based on their morphology. Epithelial cells appeared spherical with diameters of 5 - 20 microns whereas taste cells appeared as long (50 micron) and narrow (5 micron) pencil-shaped cells. Occasionally a group of taste cells could be seen still joined by the tight junctions at the apical end with the basolateral ends freely dissociated from one another. Cells were maintained at 37° on the stage of an inverted microscope for recording in L15/glucose medium. Standard patch clamp recording techniques were employed to achieve voltage clamp records in the whole-cell configuration. Cells were held at negative 65 millivolts and stepped at positive 10 millivolt increments. Transient inward followed by sustained outward currents (both up to several hundred picoamps) were recorded. Pharmacologic identification of the ionic components of these currents is in progress.

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Constraints on Transduction Mechanisms from Analysis of Olfactory Receptor Currents. STUART FIRESTEIN and GORDON M. SHEPHERD, Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

Contradictory evidence has been presented concerning the mechanisms underlying odor responses in olfactory receptor neurons. This has led to speculation that many odor responses are non-specific or are not receptor mediated. We have addressed this problem by analyzing odor induced currents in patch clamped salamander receptor neurons. The cells are mechanically isolated from the epithelium and are subjected to brief pulses and steps of a mixture of 3 common odorants. We find that 45% of the cells do not respond to odor stimulation even though they appear morphologically and electrically normal. Among responding cells the threshold odor concentration and the maximum sensory conductance vary over a wide range. These results suggest that olfactory receptor cells possess odor specific responses. Odor pulses directed at specific regions of the receptor neuron show that the odor response is confined to the ciliary and distal dendritic regions of the cell. Thus the site of transduction is spatially localized. The responses consist of a net inward current with a characteristic sigmoidal shape, monotonic rise to peak, and kinetics which include a latency of from 150 to more than 500 msec. The singular shape of the responses combined with the range of response intensities suggest a common underlying mechanism which is differentiated for different cells and ligands. Isolated cells maintained in a Ringer's bath from which all mucus has been washed away are competent for odor responses, suggesting that odor molecules act directly on olfactory cell receptors. The simplest model consistent with these results is that olfactory transduction occurs through a mechanism involving ciliary membrane receptors which bind odor molecules as ligands. We postulate that this mechanism shares similarities with receptor-ligand interactions underlying slow synaptic responses of some neurons. Supported by NS 10174, NS 07609, ONR N00014-K-0145, and R. H. Wright Fund.

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Histamine Induced Modulation of Lobster Olfactory Neurons Mediated by a Ligand-gated Channel. T.A. BAYER, T.S. McCLINTOCK, U. GRUNERT and B.W. ACHE (Whitney Lab and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL).

Olfactory (antennular) receptor cells in spiny and clawed lobsters were suppressed by the biogenic amine, histamine (HA). Selectively applying HA to the soma reversibly suppressed both the spontaneous and odor evoked activity of virtually every cell tested. The effect was graded, reversible, specific to HA and had a threshold between 0.1 and 1 μM. The effect was reversibly blocked by the HA receptor antagonist, cimetidine, and the nicotinic antagonist, d-tubocurarine, but not by the HA receptor antagonists, pyrilamine and burimamide. HA increased the conductance of the soma to Cl^-, which would presumably clamp the membrane potential near rest, thereby explaining the observed suppression of spiking. HA acted by directly gating a chloride channel: gating did not require activation of GTP-binding proteins, energy in the form of ATP, or calcium influx. These results collectively suggest that lobster olfactory receptor cells are targets of a regulatory or feedback process and implicate a novel HA receptor in this process.

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Hyperpolarizing Receptor Potentials: Implications for Multiple Transduction Mechanisms and Mixture Suppression. TIMOTHY S. McCLINTOCK and BARRY W. ACHE (The Whitney Laboratory and Depts. of Zoology and Neuroscience, University of Florida).

Olfactory receptor cells of the American (clawed) and Florida (spiny) lobster were hyperpolarized, as well as depolarized, by focally applying odorants to their dendrites. In the American lobster 10 cells were hyperpolarized and 6 were unaffected by a mixture of 100 μM L-proline, L-arginine, and L-cysteine. In the spiny lobster 1 cell was hyperpolarized, 1 was depolarized, and 6 were unaffected by the same mixture. The hyperpolarizations were graded with odor intensity. All cells tested were also depolarized by a broad spectrum odorant (fish food extract). In two cells large depolarizations evoked by the broad spectrum odorant were virtually eliminated by simultaneous application of the amino acid mixture. Focally applying the amino acid mixture to 5 isolated somata (from the American lobster) voltage-clamped at -60, -30, and -10 mV failed to evoke any response. The ability of single receptor cells to produce responses of opposite polarity suggests the existence of at least two transduction mechanisms which differ in either the species of ionic conductance altered or the biochemical mechanism by which a single conductance is regulated. Alternative explanations invoking bidirectional regulation of the initial effector molecule (e.g. a G-protein) of a single transduction mechanism lack precedent and therefore seem less likely. As proline, arginine, and cysteine have previously been identified as suppressive components of odor mixtures (Derby and Ache, 1984, Chem. Senses 9:201), the ability of the amino acid mixture to hyperpolarize the cells offers an additional, non-competitive mechanism to account for peripheral mixture suppression in these cells (Ache, Gleeson and Thompson, 1988, Chem. Senses 13:425).

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Capsaicin Desensitization on the Tongue at Low Concentrations. BARRY G. GREEN (Monell Chemical Senses Center).

One of the intriguing sensory properties of capsaicin is its ability to produce desensitization: prior exposure to the chemical can render the epithelium temporarily insensitive to subsequent exposures. In the past, desensitization had been demonstrated only when capsaicin was presented—usually over a period of several hours or days—in high concentrations (ca. 1.0%) that produced intense pain. The present study demonstrates that desensitization of a localized area of the tongue can occur after as few as 5 exposures within a 5-minute period at a nominal concentration of only 3 ppm. Stimulation of the anterior tongue was achieved by applying a 1.27 cm^2 disk of filter paper impregnated with a 3-ppm solution of ethanol and capsaicin. The filter paper was allowed to dry, then wetted with a drop of dH_2O before placement on the tongue. After 25 sec the subject rated the intensity of irritation and after 30 sec the paper was removed. This procedure was followed once per minute until 1, 5, 10 or 25 conditioning stimuli had been applied. No water rinses were allowed between stimuli. A test stimulus was delivered to the same spot on the tongue fifteen minutes after the last conditioning stimulus. As measured by the perceived intensity of the test stimulus, desensitization was virtually complete in the 5-, 10- and 25-exposure conditions. A key feature of desensitization was that it appeared only after stimulation had been interrupted for a period of several minutes; when stimuli continued to be applied at the rate of 1/min for up to 25 min, perceived irritation increased rather than decreased (i.e., sensitization occurred). These findings carry implications for the nature of the neurochemical mechanism responsible for desensitization, and perhaps for the "adaptation" to chili pepper that takes place among frequent consumers of "hot" spices. In addition, by demonstrating that desensitization can be produced so readily, the results pave the way for studies of cross-desensitization between capsaicin and other oral stimuli, including tastes.

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On the Dynamics of Odor Detection. WILLIAM S. CAIN (John B. Pierce Foundation Laboratory and Yale University) and JANNEANE F. GENT.

Thirty-two subjects between 22 and 59 yr yielded odor detection thresholds for four odorants (1-butanol, isoamyl butyrate, pyridine, and phenylethylmethylethyl carbinol) in each nostril over four sessions. The reliability of the data increased both within and between sessions, revealing a continuous influence of practice. Not surprisingly, therefore, thresholds also decreased progressively over sessions. A high intercorrelation of thresholds from one odorant to another and the impressive stability of a subject's relative position within the threshold distributions for the four odorants showed that a general factor of sensitivity dominated the outcome almost entirely. Age contributed strongly to intersubject variation. Even within this sample of nonelderly individuals, it accounted for half the variation in performance, up to two orders of magnitude in threshold. Such a strong influence became evident only through repeated testing. Other important factors included superior sensitivity in the right nostril and a negative correlation between the mean and variance of the threshold distributions. This correlation has relevance to the phenomenon of specific anosmia as commonly measured. Scant attention to the correlation may have contributed to overestimation of the frequency and specificity of the phenomenon. A clinically relevant outcome was that measurement of threshold for diagnostic purposes can generally rely on just one odorant, but that the reliability of a threshold measurement cannot be taken for granted. The time available in the clinic will place serious limitations on reliability.

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Perceptual Integration of Tertiary Taste Mixtures

ROBERT L McBRIDE (Sensory Research Centre, CSIRO Division of Food Processing, Sydney, Australia).

Integration psychophysics was used to explore the taste perception of mixtures of sucrose, fructose, and citric acid. Three levels of each stimulus were varied in a 3 x 3 x 3 factorial design. Subjects rated total intensity, sweetness, and acidity of the 27 mixtures on graphic rating scales. Consistent with earlier work, the perceived total intensity of the tertiary mixtures was found to be dictated by the intensity of the (subjectively) stronger component alone (i.e., either the integrated sweetness or the acidity, whichever was the more intense). In contrast, the sweetness and acidity of the mixture were susceptible to mutual suppression: Sweetness suppressed acidity, acidity suppressed sweetness. But there was a difference between sucrose and fructose in their interaction with citric acid, fructose being the more susceptible to suppression. This selectivity of suppression indicates that the two sweetnesses could not have been inextricably integrated, and it has implications for taste coding. The findings might be reconciled in terms of two separate coding mechanisms: one for taste intensity, another for taste quality.

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A Binary Mixture Model Applied to the Sweetness of Fructose and Glucose Mixtures: De Graaf and Frijters Revisited

DANIEL M. ENNIS (Philip Morris Research Center, P.O. Box 26583, Richmond, VA 23261).

Assuming that two substances (for example, tastants or odorants) share a common type of receptor, a binary mixture model is derived from the equations for the equilibrium constants for the separate and combined reactions of the substances and the hypothesized receptor(s). It is assumed that a multimolecular interaction between stimulant molecules and a receptor or between a stimulant molecule and several receptors may occur forming a stimulant-receptor complex. This model provides a significantly superior fit to the sweetness data of De Graaf and Frijters (Chem. Senses, 11, 295-314, 1986) on mixtures of fructose and glucose than an alternative model based on Beidler's theory of taste stimulation applied to mixtures (Prog. Biophys. Biophys. Chem., 12, 109-151, 1962). (In Beidler's model a unimolecular interaction is assumed between a stimulant molecule and a receptor). Estimates of the parameters of the model provide information on the relative stoichiometric coefficients of the reactants and the relative equilibrium constants for the reactions between the receptor and the substances alone.

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A New Method for Studying Taste Discrimination and Preference in Preterm Infants. TERESA R. MAONE, RICHARD D. MATTES and GARY K. BEAUCHAMP. (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA).

A method was developed using a gelatin-based nipple flavored with sucrose for administering a tastant to preterm and term infants. Previous methods, which measured nutritive sucking for flavored solutions, are problematic for preterm infants whose sucking is not well coordinated with swallowing. Sucking behavior elicited by the flavored gelatin nipple was compared to that elicited by a rubber nipple (without delivered liquid).

Subjects were 12 preterm infants (birth weight: 1250 g, test weight: 1945 g, test age: 36 weeks post-conception) and 24 term infants (birth weight: 2998 g, test age: 26 hr). For the preterm infants, the flavored gelatin nipple elicited stronger sucks (i.e., a greater area under the time intensity curve describing the sucking responses) than did the control nipple. For the term infants, the flavored gelatin nipple stimulated stronger and more frequent sucking with greater areas and higher trace amplitudes compared with the control nipple (see Table).

	Preterm		Term	
	Gel	Ctrl	Gel	Ctrl
# Sucks (2.5 min)	52±41	37±30	80±67	46±60*
Height (mmHg)	138±71	100±52	176±79	116±65***
Area (mmHg x sec)	42±20	32±21**	75±38	45±19***

*p < .02; **p <.01; ***p < .001.

The new flavored gelatin nipple is an effective means for studying taste in preterm and term infants. It could be adapted to contain other tastants and/or odorants. Finally, it can be used to routinely deliver sweet tastes to preterm infants which may promote the development of sucking and increase their growth efficiency.

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Olfactory Function in Chemical Workers Exposed to Acrylate and Methacrylate Vapors BRIAN S. SCHWARTZ (Clinical Epidemiology Unit, Section of General Internal Medicine, Department of Medicine, University of Pennsylvania), RICHARD E. FRYE, RICHARD L. DOTY (Smell and Taste Center, Hospital of the University of Pennsylvania, and Department of Otorhinolarygnology and Human Communication, School of Medicine, University of Pennsylvania), CARL MONROE, and SUE BARKER (Rohm & Haas Company, Philadelphia, PA).

An investigation of the olfactory function of 731 workers at a chemical facility which manufacturers acrylates and methacrylates was undertaken using the University of Pennsylvania Smell Identification Test (UPSIT). In a cross-sectional analysis of the data, no significant associations of chemical exposure with UPSIT test scores were observed. However, a nested case-control study designed to evaluate the cumulative effects of exposure on olfactory function revealed elevated crude exposure odds ratios of 2.0 for all workers and 6.0 for workers who never smoked cigarettes (respective 95% confidence intervals: 1.1 - 3.8 and 1.7-21.5). Logistic regression analysis, adjusting for multiple confounders, revealed exposure odds ratios of 2.8 and 13.5 in these same respective groups (95% confidence intervals: 1.1-7.0 and 2.1-87.6), and a dose-response relationship between olfactory dysfunction and cumulative exposure scores (semi-quantitative indices of lifetime exposure to the acrylates). The data also revealed decreasing exposure odds ratios with increasing duration since last exposure to these chemicals, suggesting that the effects may be, at least in part, reversible.

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Sensory preferences for sugar and fat among normal-weight dieters: The ice cream study. ADAM DREWNOWSKI, MELISSA SCHWARTZ and MARIA SPADA (University of Michigan School of Public Health).

The consumption of palatable foods containing sugar and fat has been linked to problems with weight control in obesity and eating disorders. However, only a few studies have examined sensory responsiveness to sugar/fat foods as a function of weight-related attitudes and behaviors. The present subjects were 79 female college students (mean age 19.8 yrs) of normal body weight (mean wt: 58.1 kg; body mass index 21.5). The average subject wished to lose 4.3 kg, and twenty-five subjects (32%) reported dieting to lose weight at the time of the study. Mean dietary restraint score for the group was 17.7 (range 5-33). Taste stimuli were 9 specially prepared ice creams containing 3 levels of sugar (12, 15 and 18%) and 3 levels of fat (10, 15 and 20%) and composed only of fresh cream, non-fat dry milk solids, sucrose, water, and 0.25% stabilizer-emulsifier mixture. The subjects rated sweetness, perceived fat content and overall acceptability of each product on 9-point category scales. Significantly higher hedonic ratings were obtained for ice-cream stimuli than for a set of sucrose solutions (range 2-32%) in distilled water. Peak preferences were obtained for stimuli containing 15% sugar and 15% fat. Subjects were then separated by hedonic response type (Type I or II), according to their relative preferences for sugar or fat. A comparison of respondents who gave respectively highest (n=13) and lowest ratings (n=16) for high fat/high sugar ice creams showed that the former tended to be heavier (63.0 vs. 57.5 kg), had higher triceps skinfolds (20.1 vs. 17.6 mm) and higher dietary restraint scores (20.1 vs. 17.7). However, persistent dieting and extremely high restraint scores were linked to enhanced fat content scores and lower preferences for high-fat ice creams. The findings that some normal-weight dieters report a dislike of high-fat foods are consistent with clinical studies on fat avoidance in eating disorder patients.

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Temporal Patterns of Citric Acid Taste Intensity. S.T. KELLING[1], K. LYKE, B.P. HALPERN[1,2] (Physiology[1], Psychology & NBB[2], Cornell University, Ithaca NY, 14853-7601 USA)

Magnitude estimates [ME] of taste intensity increased with stimulus duration for continuous [C] 50-2000 ms 2mM sodium saccharin [NaSac] (K & H, Chem. Senses, 1988) but 5-10 Hz train ME asymptoted before 2000 ms (ISOT IX, 1987). RT of C ME were 1656-2106 ms. Tracked [100 ms resolution] intensities [T-INT] of NaSac did the same (K, Maney, & H, Chem. Senses, 1988), but RT for >0 T-INT were 700-800 ms. We had five subjects track 10-30mM C & 2.5-5 Hz citric acid solutions [CA] at cumulative solution durations [CSD] of 800-2000 ms (= 1600-4000 ms total stimulus durations for trains) delivered to the anterodorsal tongue (K & H, Chem. Senses, 1986). RESULTS: Overall T-INT for 10mM at each duration differed significantly from others [p<.0004] except 1000 ms 2.5 vs 5 Hz [p=.14]; for 30 mM, all at 800-1000 ms [p<.002] except 800 ms 2.5 vs 5 Hz [p=.14] but none at 2000 ms [p>.06]. CSD was significant [p<.02] for overall T-INT except 10mM 5 Hz 800 vs 1000 ms; concentration, always significant [p<.0003]. Median RT for 10mM >0 T-INT was 1100 ms; for 30mM, 1000 ms. Maxima, time of maxima, and time of T-INT return to 0 increased with CSD. Train maxima exceeded C maxima except for 30 mM 2000 ms CSD C vs all trains. CONCLUSIONS: Intensity of CA trains increases with CSD. For T-INT, the CA time of maximum is a function of CSD ; maximum T-INT, f(CSD, frequency, and concentration); time of return to 0, f(CSD, concentration). In contrast to NaSac, CA train maxima often exceed C maxima. The temporal pattern of taste intensity is dependent upon stimulus molelcule, concentration, duration, and frequency.

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Distribution of Radioactive Leucine in Crayfish Olfactory Lobes Following Uptake and Transport by Restricted Numbers of Primary Sensory Neurons.

DEF. MELLON, (Department of Biology, Univ. Virginia, Charlottesville)

In the crayfish, Procambarus clarkii, exposure of external antennular filaments to tritiated leucine (60 Ci/mm) for two hours results in uptake and transport of the labeled amino acid to the glomeruli of the ipsilateral olfactory lobe (Mellon, Tuten, Redick, J. comp. Neurol., 1989). When all of the 150-175 antennular aesthetascs are exposed to the tritiated leucine, intense, uniform labeling of the glomeruli results. In order to investigate the projection patterns of primary olfactory sensory neurons to the olfactory lobe glomeruli, we have restricted the exposure of tritiated leucine to small groups of aesthetascs on different parts of an antennular filament. If olfactory sensory axons do not branch centrally to more than one target glomerulus, the distribution of labeled glomeruli should reproduce the projection pattern of individual sensory neurons associated with the exposed aesthetascs. Using autoradiographic analysis, we compared the distribution of label in olfactory lobes of animals that had restricted or total exposure to radioactive leucine. Total exposure of an antennular filament generated intense, generalized labeling of the ipsilateral olfactory lobe glomeruli. Restricted exposure generated weaker labeling, but the distribution of label in the affected olfactory lobes was as generally distributed as in the totally exposed cases. We conclude that axonal projections from only two or three aesthetascs (350-525 sensory neurons) are widely distributed within the ipsilateral olfactory lobes. Encompassed within this broad pattern of projection is the possibility that the neurons associated with any one aesthetasc distribute their central terminals to all available olfactory lobe glomeruli.

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Immunohistochemical and Biochemical Evidence for Histamine and GABA in the Olfactory Lobes of the Spiny Lobster. E. ORONA, B-A. BATTELLE, AND B. W. ACHE. (Whitney Laboratory and Depts. of Neuroscience and Zoology, University of Florida, St. Augustine, FL. 32086)

The axons of lobster olfactory receptor cells synapse with second-order neurons in the glomerular neuropil of the olfactory lobe (OL). As an initial effort to investigate inhibitory interactions in the OL, we attempted to identify histamine (HA) and gamma-aminobutyric acid (GABA), putative inhibitory neurotransmitters in crustaceans, in the OL of the spiny lobster. Polyclonal antibodies (Chemicon) raised against HA and GABA were used to localize the antigens in sections of the brain. Both HA- and GABA-like immunoreactivities were found using avidin-biotin conjugated peroxidase. Control staining was negative and included omission and preabsorption of the primary antibody. Biochemical methods were used to confirm the specificity of the immunohistochemistry. The OL tissue was shown to be capable of synthesizing HA from radioactive histidine. (Similar experiments with GABA are in progress.) Positive HA- and GABA-like immunoreactivity was found in adjacent clusters of the cell bodies of olfactory interneurons. Intense glomerular staining for both HA and GABA was also found in the OL, with a predominance of staining in the outer caps of the glomeruli, which are thought to be the regions where the primary afferent terminals contact the processes of olfactory interneurons. Our findings implicate inhibition at the first synaptic level of the olfactory pathway and suggest that the glomerulus is a potential locus of central mixture suppression.

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Olfactory Receptor Axons Run in Parallel on the Lateral Face of the Rat Olfactory Bulb. DAVID P. WELLIS and JOHN W. SCOTT (Deptartment of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322)

Previously we suggested that odor-induced responses of rat olfactory bulb (OB) output neurons may be mediated through bulbar interneurons as well as through direct contact from receptor cell axons. Selective removal of the direct sensory contact from a population of output neurons would more directly establish the role of interneuronal inputs to these cells during olfactory processing. The projection from the olfactory epithelium to the OB shows some topography, but the degree of precision of this projection, especially as the fibers traverse the surface of the bulb, is unknown. Freeman (1974) showed these fibers to be organized in a parallel manner in the cat and rabbit. We evaluated the discreteness of the projection after the fibers pierced the cribiform plate by making small cuts into the olfactory nerve layer (ONL) on the lateral face of the OB. The resulting denervation was evaluated both anatomically, applying WGA-HRP to the nasal epithelium by the method of Shipley (1985), and physiologically, measuring electrically-induced olfactory nerve field potentials. Rostral to the nerve cut, the ONL and glomerular layer (GL) showed intense labelling around the entire circumference of the bulb. Transneuronal labelling of the mitral cell layer (MCL) and superficial half of the external plexiform layer could be seen in animals surviving at least 3 days. However, primary and transneuronal labelling were not observed in these areas in the region caudal to the nerve cut. The region which failed to label was similar throughout all sections, showing that a small cut, down to 400 um, could induce a 'beam' of denervation along the lateral face of the OB. Similar nerve cuts rostral to the cribiform plate or on the dorsal surface of the bulb failed to produce a distinct beam of denervation. Injections of HRP and/or fast green made at the electrophysiologically-evaluated borders of the denervated region correlated well with the borders of labelling found following WGA-HRP application in animals where both types of evaluation were performed. These results show that the primary olfactory axons run in a discrete parallel fashion along the lateral face of the OB. Preliminary results from intracellular recording of output neurons in the denervated region show that these cells can respond to odor without direct contact from receptor cell axons. The denervated preparation permits a more direct test for synaptic mechanisms, such as lateral inhibition and disinhibition, involved in olfactory coding in the OB.

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The Lectin UEA1 Labels Rat Olfactory Glomeruli MARCIA J. RIGGOTT and JOHN W. SCOTT (Department of Anatomy and Cell Biology, Emory University, Atlanta, GA 30322)

Lectins and antibodies to the carbohydrate portions of cell surface glycoproteins have been useful markers for subsets of neuronal cell bodies and their processes. We report that the lectin Ulex europaeus 1 (UEA1) used at a concentration of 0.01% demonstrates high affinity for the afferents and glomeruli of the accessory bulb and for a large subset of afferents and glomeruli of the main olfactory bulb of the adult rat. This labeling is blocked by fucose but not by monomers of N-acetyl-glucosamine, N-acetyl-galactosamine, galactose or mannose. The lectin binding specificity for fucose was evaluated using densitometric scans taken at equivalent locations within representative coronal sections. Maps constructed from these coronal sections show that in rostral regions of the main bulb, the greatest lectin binding is found within the ventrolateral glomeruli. This labeling pattern shifts ventromedially in more caudal regions of the bulb. Glomerular binding with UEA1 is greatest in the midportion of the main bulb along its rostral-caudal extent at the ventral midline. The glomeruli are heterogeneous in their expression of fucosylated glycans; those adjacent to the heavily labeled regions can be either heavily or lightly labeled with the lectin although individual glomeruli are uniformly labeled. The glomerular layer of the accessory bulb is uniformly heavily labeled by UEA1. In summary, the heterogeneous binding of the lectin UEA1 to subsets of glomeruli suggests a differential expression of fucosylated glycans in the olfactory bulb.

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Localization of Corticotropin-Releasing Factor-Like Immunoreactivity in the Squirrel Monkey (Saimiri sciureus) Olfactory Bulb and its Efferents. JULIA L. BASSETT (Univ. of Calif., San Diego), STEPHEN L. FOOTE (Univ. of Calif., San Diego) and MICHAEL T. SHIPLEY (Univ. of Cincinnati)

Biochemical and electrophysiological evidence suggest that olfactory bulb efferents to the primary olfactory cortex utilize excitatory amino acids as neurotransmitters. Other studies have raised the possibility of a cotransmitter within this system (Hori et al., 1981). Imaki et al., (in press) have demonstrated that all mitral and most tufted cells in rat are immunoreactive for corticotropin releasing factor (CRF) and contain the mRNA for this peptide. The distribution of CRF has not been examined in the primate olfactory bulb or its efferents. The present study uses immunohistochemical (IHC) methods to localize CRF in non-colchicine treated monkeys.

Sections through the olfactory bulb, anterior olfactory nucleus and olfactory cortex were processed for IHC using a polyclonal antiserum directed against the rat form of CRF (kindly donated by J. Rivier and W. Vale). Within the olfactory bulb, nearly all mitral and many tufted cells contained CRF-like immunoreactivity. CRF-positive fibers and isolated, immunoreactive puncta were seen within the olfactory tract and olfactory stria. Immunoreactivity with a fine, particulate appearance was seen around the cells and within the neuropil of the anterior olfactory nucleus. Within the olfactory tubercle and pyriform cortex, numerous immunoreactive varicose processes were seen in the superficial portion of the external plexiform layer. CRF-like immunoreactivity also was seen in olfactory recipient parts of the amygdala and in the entorhinal cortex. These observations suggest that CRF may be an important neuromodulator in the output neurons of the mammalian olfactory bulb. Physiological studies are underway to investigate this possibility.

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Comparison of Staining Patterns of Rat Olfactory Bulb Glomeruli by Cytochrome Oxidase and Acid Phosphatase. CARLA WEINBERG AND ESMAIL MEISAMI (Dept. Physiol & Biophys, Univ. of Illinois, Urbana, IL, 61801)

We are carrying out a series of histochemical studies in the olfactory bulb of normal adult rats using cytochrome oxidase and acid phosphatase staining patterns as markers for mitochondrial and lysosomal activity respectively. Both acid phosphatase and cytochrome oxidase stain the olfactory glomeruli more intensely than the adjacent olfactory nerve and external plexiform layers. However careful comparison of the staining patterns for these two enzymes reveals that across the glomerular layer, the various glomeruli do not stain uniformly, some glomeruli staining more intensely than others. Also this non-uniformity is evident even within a particular glomerulus. Based on the assumption that cytochrome oxidase reflects neural activity whereas acid phosphatase reflects neural degeneration or turn-over, we are analyzing the patterns of staining for these two enzymes in 30 um adjacent sections cut frontally or horizontally in a cryostat. These adjacent sections are then compared from photographs or by redrawing the glomeruli and the stained regions using a camera lucida. Two findings emerge from these preliminary results. First, the glomeruli staining darkly for cytochrome oxidase tend to stain lightly for acid phosphatase and vice versa. Second, when the planes of two adjacent sections can be ascertained to go through the same glomeruli, as judged by the particular morphology of the glomerulus involved, it can be seen that even within a glomerulus, areas that are cytochrome oxidase positive, tend to be acid phosphatase negative and vice versa. These results imply that in the adult rat, olfactory glomeruli show differential patterns of activity and degeneration and that furthermore this spatial variation in activity may be present even within the same glomerulus.

Supported by research funds from University of Illinois.

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Pharmacology of Inhibition in the Glomerular Layer of the Olfactory Bulb. II. Interactions with Modulatory Systems. W.T. NICKELL and M.T. SHIPLEY (University of Cincinnati).

Binding studies indicate that $GABA_b$ receptors in the olfactory bulb of the rat are confined to the glomerular layer. Consistent with this restricted anatomical localization, we reported previously (AChemS X) that topical application of a $GABA_b$ agonist, baclofen, reduces the amplitude of field potentials evoked by stimulation of the olfactory nerve. We hypothesize that this inhibitory mechanism functions to regulate the strength of the synaptic input from the olfactory nerve terminals onto mitral cell dendrites; however, characteristics of the inhibition revealed by double pulse stimulation of the olfactory nerve also indicate a $GABA_a$ mechanism located at the granule-mitral synapse in the external plexiform layer. Thus, the functional significance of the glomerular $GABA_b$ system is not clear.

The glomerular layer receives neuromodulatory inputs from the basal forebrain (acetylcholine and possibly GABA) and from the median raphe (serotonin). We have tested the effect of blocking one of these inputs by applying muscarinic receptor antagonist, atropine, to the surface of the olfactory bulb.

Following application of atropine the response to olfactory nerve stimulation is inhibited. At high concentrations of atropine the response to single olfactory nerve shocks is inhibited; at lower atropine doses the response to single shocks is not affected, but repeated stimulation at an interval of 100 msec reveals a slowly developing inhibition not present prior to atropine application.

This result could occur if tonically released acetylcholine normally blocks the expression of an inhibitory mechanism located in the glomerular layer. This blockade might result either from cholinergic inhibition of periglomerular cells or from a direct muscarinic inhibition of the $GABA_b$ response by a mechanism recently described in hippocampus (PNAS 84:3467, 1987). Interactions between the serotonergic input to the glomerular layer and $GABA_b$ or cholinergic receptors are also possible. Experiments are planned to test these possibilities.

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Patterns of Functional Projections between the Salamander Olfactory Epithelium and Bulb Visualized Using a Voltage-Sensitive Dye. A.R. CINELLI, S.N. NEFF and J.S. KAUER (Tufts Med. Sch. and New England Med. Ctr., Boston, MA.)

Video imaging of changes in olfactory bulb voltage-sensitive dye fluorescence after punctate electrical stimulation of restricted mucosal locations was used to define the spatial pattern of functional projections between these two regions. Activity patterns across and within the layers of the olfactory bulb were recorded using a video system with high spatial (256 x 256 pixels x 8 bits) and substantial temporal resolution (33 ms/frame). Distinctively different spatial patterns which included depolarization and hyperpolarization were observed depending on the site of stimulation. In general, these patterns took the form of several, simultaneously activated locations distributed within the various layers of the bulb and were consistent across animals. Widespread distributed patterns were more typical of stimulation of the dorsal olfactory mucosa than of the ventral epithelium, although stimulation of the ventral anterior mucosa elicited the most widely distributed patterns having poorly defined borders and showing substantial overlap when different sites within this area were stimulated. Increasing the intensity of stimulation of this anterior region produced a increase in the size of the area of activation. This is in contrast to the caudal, dorsal epithelial region where an increase in stimulus intensity elicited little change in the size of the activated area.

Examination of the relationships among these spatially distributed patterns provides additional support for the hypothesis that there are complicated, divergent and convergent projections from the mucosa onto the bulb, and that these connections may be important for understanding how odor stimuli are encoded and manipulated by the olfactory pathway.

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Long-lasting Depolarization of Mitral/Tufted Cell Dendrites in the Salamander Olfactory Bulb are Related to Ca++ Influx. A.R. CINELLI, K.A. HAMILTON and J.S. KAUER (Tufts Med. School and New England Med. Ctr, Boston, MA.)

Using a variety of voltage-sensitive dyes, we and others have observed a relatively long lasting (100-300 ms) depolarization in the vicinity of the external plexiform layer in the olfactory bulb after both electrical and odor stimulation. In contrast to the rapid, short latency depolarization that seems to be related to synchronous activity in the olfactory nerve or in mitral/tufted (M/T) somata and axons, the origins of this slower potential are less certain. Others (Jahr and Nicoll, J. Physiol., 1982; Mori et al.,J.Neurosci.1982, 1984) have suggested that the fast signals they have seen in this region may represent Ca++ currents related to depolarization of the M/T dendritic arborization.

To examine currents within the dendritic arborizations of identified bulbar cells, we have employed real-time video imaging of cells individually injected with the calcium sensitive dye FLUO-3 or with voltage sensitive dye. These methods has shown that electrical stimulation of the olfactory nerve gives rise to non-uniform, relatively long-lasting FLUO-3 signals distributed across the dendritic arborization of mitral/tufted cells. These results provide additional evidence to support the hypothesis that Ca++ currents accompany the late depolarization observed with voltage sensitive dyes (Cinelli and Salzberg, Neurosci. Soc., 1988), and that these events may relate to the spatial distribution of activity patterns generated by different odors. Further analysis using this approach should provide important cues for understanding how the processing of information may occur in dendrites.

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Glomerular Layer Modulation of EEG and Evoked Potentials In the Rat Olfactory Bulb. Paul B. COOK, Barry K. RHOADES, and Walter J. FREEMAN (University of California at Berkeley).

The bulbar EEG alternation of inspiratory cyclic bursts and chaotic interburst activity and the damped sinusoidal oscillation seen in averaged evoked potentials (AEPs) and unit post-stimulus-time histograms (PSTHs) are best accounted for by modelling the bulb as a set of coupled oscillators. The negative feedback relationship of the reciprocal synapses between mitral and granule cells in the external plexiform layer establishes these oscillators. Electrophysiological evidence indicates two modulatory effects of glomerular layer interneurons on these "deep" oscillators - a dynamic range compression of glomerular throughput and a modulation of mitral cell excitability The neurochemical basis for these effects was investigated by application of neuroactive substances acting on GABA (an intrinsic neurotransmitter of the periglomerular and granule interneurons) and histamine (a probable extrinsic neuromodulator) receptors.

Rats were prepared for electrophysiological recording by pentobarbital anesthesia supplemented with analgesia at surgical and stereotaxic sites. The lateral bulbar surface, primary olfactory nerve (PON) fiber bundles of the olfactory mucosa, and the lateral olfactory tract (LOT) were unilaterally surgically exposed. The PON and LOT were stimulated via bipolar electrodes. EEG and evoked potentials (EPs) were recorded from a linear array of surface electrodes oriented dorso-ventrally on the lateral bulbar surface, while the EEG of the contralateral bulb was recrded via an electrode on its dorsal surface. Multiunit PSTHs were recorded from the mitral cell layer via a tungsten microelectrode. EPs and PSTHs were recorded for near threshold stimulus intensities and averaged over 50-100 trials. All neuroactive substances were administered locally to the bulbar surface in the vicinity of the recording array via a push-pull cannula system.

Application of picrotoxin or muscimol radically alters the frequency and amplitude of the AEP oscillation, indicating a direct effect on the GABAergic granule-mitral synapses which overwhelms any glomerular layer effect. The GABAB agonist baclofen elevates PON threshold, while markedly increasing the amplitude of the slow negative wave AEP component. Baclofen decreases the damping of the LOT AEP and increases EEG burst activity. The GABAB antagonist phaclofen reverses these effects. Electrophysiological results indicate a role for GABAB receceptors in presynaptic inhibition of glomerular layer GABAA synapses. Histamine produces continuous bursting in the EEG, dramatically decreases damping of the LOT AEP, and attenuates both the sinusoidal and slow negative wave PON AEP components. This suggests a centrifugal excitatory role for histamine on glomerular layer activity. PSTHs for both baclofen and histamine applications demostrate the 1/4 cycle phase lead of unit spikes (mitral activity) over EEG (granule activity) predicted by the coupled oscillator model.

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Sensory and Perceptual Codings in Olfaction

WALTER J. FREEMAN (University of California at Berkeley)

Olfactory information exists in two codings in the bulb and cortex. Both forms are spatial, in that the information is presented in spatial patterns of action potentials for the time duration of sniffs or inhalations. (1) Receptor induced information that is specific to each odorant is carried by neurons that are excited or inhibited selectively by stimuli. The sensory activity is discretized to particular pulses of particular neurons. (2) The perceptual information that is specific to an odor is triggered by the stimulus and shaped by (a) synaptic weights embodying past experience and (b) neural excitability set by centrifugal tracts expressing factors of arousal and expectation. The information is distributed spatially with uniform density. All the olfactory neurons participate equally in the expression of every odor, by fast or slow pulse rates or by no pulses at all during a sniff. The sensory information is converted to the perceptual form of information by state transitions in the bulb that are observed in the form of bursts of impulse and EEG activity that accompany inhalations. Each oscillatory burst is characterized by a well defined phase gradient over the surface of the bulb, having the form of a cone in spherical coordinates. The apex marks the site of nucleation for the transition from interburst to burst. It varies randomly and carries no behavioral information. The fraction of activity of single neurons that is contributed to the whole is on the order of 1 part in 1,000-10,000. Hence with present technique limiting multiunit simultaneous recording to less than 100 cells the perceptual code cannot be read with multiunit recordings. Using time ensemble averaging for PSTH and AEP is impermissible. Correspondingly, an EEG recording is not suited to observing the sensory information. Thus unit and EEG recording techniques are fully complementary.

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Differential Expression of Microtubule-Associated Proteins 1A and 1B During Postnatal Development of the Olfactory Bulb. THOMAS A. SCHOENFELD (Clark Univ.) AND RICHARD B. VALLEE (Worcester Found. for Exper. Biology)

A panel of monoclonal antibodies to high molecular weight microtubule-associated proteins (MAPs) 1A and 1B was used to examine the distribution of these MAPs in the olfactory bulb and other prominent regions of the postnatally developing rat CNS. We find that anti-MAP 1B staining is particularly intense in the olfactory nerve at birth and persists into adulthood, whereas antibodies to MAP 1A do not stain the olfactory nerve at any age. Similarly, MAP 1B but not MAP 1A is associated with axonal outgrowth in parallel fibers of the cerebellar cortex, mossy fibers of the hippocampus and the corticospinal tract at appropriate stages in their development. On the other hand, anti-MAP 1A staining at birth is particularly intense in the somata and proximal dendritic trunks of olfactory bulb principal and intrinsic neurons, and this also persists into adulthood. As in the cerebellum, this staining pattern appears to be associated with the older and perhaps more stable segments of developing dendrites rather than with the newer, more distal, growing tips. MAP 1B is eventually expressed in somata and dendrites but this lags behind the expression of MAP 1A in these neuronal compartments. Likewise, MAP 1A is eventually found in numerous axonal systems in the adult, but lags somewhat behind the appearance of MAP 1B in axons, although anti-MAP 1A staining is never found in the olfactory nerve. These observations underscore previous work showing both the persistent growth and/or turnover of olfactory nerve axons and the more rapid pace of olfactory bulb neuronal maturation compared with that in the cerebellum and hippocampus. Since biochemical data appear to indicate that MAP 1B has a lower affinity for microtubules than does MAP 1A, MAP 1B may confer a relative state of lability on the cytoskeleton of olfactory nerve axons that may be critical to their capacity for rapid and complete degeneration during the process of turnover and regrowth.

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A numerical study of mitral cells and glomeruli in the olfactory bulbs of macaque monkey and developing hamsters. ESMAIL MEISAMI, JORDAN FRISHMAN AND JULIE KIM (Dept. of Physiology, Univ. of Illinois, Urbana, IL 61801).

Detailed quantitative information regarding the number of relay elements in the mammalian olfactory system is available only for the rabbit and rat. We have been collecting data in this regard for two different species of mammals, the macrosmatic hamster and the microsmatic macaque monkey. The hamster data include a developmental study as well. Quantitative morphometric and cell count methods were applied to frontal serial sections obtained from 4-year old monkeys and from hamsters at the postnatal ages of 1, 10, 15, 25 and 90 days. The sections examined were 10 um thick and Nissl stained with cresyl violet. For the mitral cells only cells containing nucleoli were counted; to count the glomeruli, a new morphometric procedure involving determination of the total volume of the individual glomeruli by were employed. The glomeruli were identified as the spherical structures surrounded by the periglomerular cell assemblies. The number of mitral cells were found to be about 20,000 in the hamster bulb and this number remained the same at all postnatal ages although the density of the cells in the mitral cell layer decreased up to day 25. The number of glomeruli as defined above was found to be very low in the young postnatal hamster increasing throughout the postnatal period to plateau by day 25. The monkey bulbs were found to have about 18,000 mitral cells and the density of these cells in the mitral cell layer were found to be considerably smaller than that in the adult hamster. Although the glomerular layer is fairly robust in the monkey, compared to the hamster, it is less developed. Counts of the monkey glomeruli are in progress. Supported by research funds from the University of Illinois.

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Survival of Olfactory Neuron Transplants in the Brain of Old Hamsters. EDWARD E. MORRISON and RICHARD M. COSTANZO (Medical College of Virginia).

In previous studies, it has been demonstrated that olfactory neuroblasts can be successfully transplanted into the brain of litter mates and young adult host animals. Within the young brain environment, the transplants survived, developed new neurons and grew axon processes into the host brain parenchyma. The purpose of this study was to determine if neonatal olfactory neurons can survive and develop when transplanted into an "old" adult brain. Hamsters, ranging from one to two years of age, were used as host animals. Olfactory transplant tissue was obtained from the septum region of neonatal hamsters (P5-10) and placed within the parietal lobe using a glass probe. Following recovery times exceeding 60 days, host animals were anesthetized and processed for light microscopic examination.

Our results demonstrate that olfactory neurons continue to survive, develop and mature even when transplanted into an "old" brain. Olfactory transplant tissue formed vesicles that were lined by a respiratory or sensory epithelium. We observed olfactory neurons in the epithelium, lamina propria and within the host brain parenchyma. Mitotic figures were seen among the transplant neurons. Axons originating from the transplant, formed fascicles that grew into the host brain parenchyma. The results of these preliminary studies demonstrate that an old host brain can still provide a suitable environment for transplanted neurons. Our results may have implications for studies concerning the neuronal aging process and related diseases.

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Olfactory Nerve Input Induces Tyrosine Hydroxylase Immunoreactivity in Rat Forebrain Neurons. KATHLEEN M. GUTHRIE AND MICHAEL LEON (Dept. of Psychobiology, University of California, Irvine CA 92717)

Functional olfactory nerve input is required for the normal expression of tyrosine hydroxylase (TH) by dopaminergic neurons in the glomerular region of the rodent main olfactory bulb. To determine whether olfactory nerve input exerts a similar influence on cells in other brain regions, we allowed the olfactory nerve to innervate the rat forebrain. To accomplish this, we performed unilateral bulbectomies in rat pups on postnatal day 5-7 and examined the brains 2-6 months later, after the regenerated olfactory nerve fibers had penetrated the forebrain. Tissue was stained for TH-, dopamine B-hydroxylase (DBH)-, and olfactory marker protein (OMP)-immunoreactivity. We observed TH-immunoreactivity in neurons located in areas of the adult forebrain which received olfactory nerve input, particularly along the rostral extension of the subependymal layer. This is the first demonstration of novel neurotransmitter expression in the adult brain. Many of these neurons resembled the periglomerular cells of the olfactory bulb. No cell staining for DBH-immunoreactivity was observed in these areas, suggesting the possible dopaminergic phenotype of these neurons. TH-immunoreactive neurons were also observed among granule cells surrounding ectoptic glomeruli which formed after partial bulbectomy. These results are particularly interesting because subependymal cells give rise to both periglomerular and granule cells during bulb development, suggesting that the transmitter phenotype of these interneurons is ultimately influenced by proximity to the olfactory nerve.

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Angiogenesis in the Olfactory Bulbs of Normal and Unilaterally Odor Deprived Rats. DONNA L. KOROL and PETER C. BRUNJES, University of Virginia, Charlottesville, VA 22903

Closure of a single external naris on the day after birth (P1) results in reductions in glucose metabolism and protein synthesis in the ipsilateral olfactory bulb as early as P2. Deprived olfactory bulbs also exhibit decreased size with changes in some laminae emerging as early as P8. These robust changes have been interpreted as resulting from diminished levels of afferent information handled by the developing bulb. Decreases in the amount of growth may subsequently induce changes in the development of bulb vasculature. Alternatively, the decrease in afferent activity may result in decreased angiogensis, which in turn causes a slowing of bulb growth. In this study, angiogenesis was examined in rat pups that underwent either unilateral naris closure or sham surgery on P1. At several postnatal ages pups were injected i.c. with WGA-HRP, the brains removed, fixed and subjected to TMB histochemistry. Vasculature was measured with an image analysis system which determined amount of staining per unit tissue area. Two gradients were noted: superficial to deep and caudal to rostral. Highest densities were noted in the external bulb layers (glomerular (GLM), external plexiform (EPL), mitral and internal plexiform layers). A similar outside-in gradient was observed within this external region. Vascularization also increased in caudal bulb locations. In animals reared until Day 30 deprived bulbs exhibited 10% and 25% less staining per unit area in the GLM and EPL, respectively, when compared to the contralateral control side. Staining in the granule cell zone appeared unaffected. Deprived bulbs are therefore not merely scaled down versions of normal bulbs: they contain less vasculature per area. Whether changes in vasculature occur before or after alterations in bulb size is presently being studied.
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Development of the Anterior Olfactory Nucleus in Normal and Unilaterally Odor Deprived Rats. JESSICA L. BROWN and PETER C. BRUNJES, University of Virginia, Charlottesville, VA 22903.

Rat pups with one external naris closed on the day after birth (P1) exhibit 25% decreases in the size of the ipsilateral olfactory bulb by P30. The decrease is the result of a cascade of changes which include alterations in bulb cell number, laminar volume, metabolic activity, protein synthesis and neurotransmitter expression. Large changes in bulb growth such as these suggest that deprivation-induced changes may also occur in higher order olfactory structures. In this paper we examine the development of the anterior olfactory nucleus (AON), the first synaptic target of bulb efferents. Rat pups underwent either unilateral naris occlusion or sham surgery on P1 and were sacrificed at either P10, 20 or 30. Laminar volumes were calculated using serial section planimetry for pars dorsalis, ventro-posterioralis, lateralis, medialis and externa, as well as the lateral olfactory tract and subependymal zone. No differences were found in AON subregion sizes in animals examined at P30, suggesting that deprivation does not have as significant effects here as in the bulb. Several metabolic indices were then examined to determine if deprivation had more subtle effects. A densitometric analysis of histochemical staining for the Kreb's cycles enzyme succinate dehydrogenase in pars lateralis revealed no differences between deprived and control sides in 2 P20 subjects. To date we have examined 1 animal injected with ^{3}H 2-DG on P20 and found that glucose uptake is lower in the pars lateralis ipsilateral to the occluded naris. No right/left differences were encountered in control animals for any measure. Therefore, while the effects of deprivation in the AON appear to be milder than those seen in the bulb, some changes are observed. The relative immunity of the AON may stem from the complex nature of its inputs which include substantial contributions from contralateral olfactory structures.
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In Search of the Odor Memory Engram: Thalamocortical and Paleocortical Loci. BURTON M. SLOTNICK and JUDITH M. RISSER (The American University)

Two main targets of the olfactory system, the posterior piriform cortex/amygdala/entorhinal cortex (olfactory paleocortical projections) and the prefrontal orbital cortex (olfactory neocortical projection) were deafferented by transection of the posterior lateral olfactory tract (group LOT) or by destruction of the thalamic mediodorsal nucleus (group MD) in rats trained preoperatively to discriminate among 8 different odors. Postoperative tests conducted under extinction (no reinforcement for correct responding) demonstrated excellent retention in controls and both lesioned groups. However, rats with combined LOT and MD lesions had little or no retention. These results suggest that both paleocortical and neocortical sites participate in long-term odor memory storage and that they are equipotential for this function.

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Odor Memory in the Rat. ALEJANDRA J. PAZOS and BURTON M. SLOTNICK (The American University)

To investigate capacity for long-term memory of odors four rats were trained in a multiple-channel olfactometer to discriminate among 8 different odors presented in random order within a training or test session. When animals achieved criterion performance (80% or higher correct responding on each odor), they were rested in their home cages for 6 weeks (two rats) or 8 weeks (two rats) and then tested for retention of the 8-odor task but with the significance for one S+ odor and one S- odor (a different pair for each rat) reversed.

Each rat had excellent retention of the 8 odors. On the non-reversed odors accuracy was 90% or greater. On the reversed odors each animal performed at chance i.e., they responded to the previous S+ odor and inhibited responding to the previous S- odor. The retention of three additional rats tested after a 10 week interval was more variable. One had virtually perfect retention (97%) but the other two scored 72 and 76 percent correct respectively. These results demonstrate that rats can maintain memory for 8 odors for at least 6 to 8 weeks. Additional studies will be required to explore the full capacity of rats to learn and remember odor stimuli.

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Recognition and Memory for Propionic Acid Vapor in Rats with Lesions of Olfactory Bulb Areas associated with 2-DG Uptake. XI-CHUN MAY LU and BURTON M. SLOTNICK (The American University)

Using 2-DG, a discrete focus of metabolic activity in the dorsomedial aspect of the olfactory bulb occurs in rats exposed to propionic acid vapor (PA) (Bell et al., Brain Res., 1987). However, destruction of this bulbar area is without effect on detection of or sensitivity to PA or ability to discriminate PA from other odors (Slotnick et al., Brain Res., 1987; unpublished results). To test if this bulbar area plays a role in odor recognition rats were trained to respond to PA and (as a control odor) ethyl acetate (S+ stimuli) but not to a wide variety of other odors (S- stimuli). If destruction of the PA focal area disrupted recognition then experimental rats should continue to respond to ethyl acetate but not to other odors, including PA. The critical postoperative test was conducted under extinction to eliminate biasing effects of reinforcement. Results demonstrated that rats with the PA focal area removed, like controls, had good recognition of PA. Thus on the postoperative test all rats responded to PA but inhibited responding to other new odors (including acetic acid, an odor similar to PA). In subsequent tests, experimental rats performed as well as controls in discriminating between PA and acetic acid and in detecting PA in a mixture of PA and acetic acid. In summary, these experiments fail to provide evidence for a role in behavior for an olfactory bulb area associated with high 2-DG uptake.

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Morphological analysis of the interneurons of the accessory olfactory bulb.

SHIGERU TAKAMI & PASQUALE P.C. GRAZIADEI (Department of Biological Science, Florida State University, Tallahassee, Florida)

Previous observations have shown that the rat accessory olfactory bulb contains at least five neuronal types. Types I and II have been demonstrated to be output neurons by means of WGA-HRP retrograde labeling from the medial amygdaloid nucleus; types III, IV and V are putative, local interneurons (Takami & Ichikawa, in preparation).

Topography and fine morphology of the putative, local interneurons will be illustrated here. Adult rats were sacrificed by transcardial perfusion of buffered aldehydes; the main and accessory olfactory bulbs were processed with the rapid Golgi method to allow both LM and TEM examination. Type III neurons are found in the periglomerular region, have a relatively small perikaryon and have sparsely branched dendrites. Type IV neurons have the soma located in the external plexiform layer and their long dendrites extend in all directions. Type V neurons are the ones that most directly resemble the granule cells of the main olfactory bulb, their soma is located in the granule cell layer of the accessory olfactory bulb and their long dendrite extends through the external plexiform layer at times reaching the glomerular layer. Most putative local neurons have their dendritic arbor studded with spine apparatuses and they lack an easily recognizable axon. Detail of the ultrastructure and synaptology of these local neurons will be illustrated.

While the morphology and topography of these neurons justify the classification as described above, further characterization of their connections and of their biochemical profile (now in progress) is necessary to understand their role in the circuitry of the accessory olfactory bulb.

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Perinatal Olfaction in the Mouse: Developmental Morphology of the Vomeronasal Canal and Preliminary 2-Deoxyglucose Studies In Utero.

DAVID M. COPPOLA & ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury MA 01545)[1]

The ability to detect chemical signals *in utero* provides obvious adaptive advantages to mammals that must rely on their sense of smell for survival immediately or soon after birth. Chemosensory information about the world outside the womb is present in the amniotic fluid, that could be used postnatally to recognize the nipple, mother, the nest, self, kin and even maternal dietary factors. Despite the dearth of studies in this area there is evidence that the ontological adaptations necessary to detect and store chemosensory information before birth exist in at least some mammals. Our studies of this problem in mice, originally centered on the Accessory Olfactory System since seminal studies of the rat using the 2-Deoxyglucose (2-DG) technique demonstrated enhanced labelling of the Accessory Olfactory Bulb (AOB) *in utero*. Our studies of stimulus access to the Vomeronasal organ in the mouse revealed that the canal connecting the sequestered receptor cells of the AOS to the nasal cavity is not patent prenatally and thus the AOS could hardly be detecting chemicals in the amniotic environment (Chem. Senses 13(4):680). Now we report the results of two studies. The first describes the developmental morphology of the VNO canal in mice at E19, D1, D5, D10, D15, D25 in 5μ plastic sections. The results confirm our previous study in which the precursor of the VNO canal was not patent on the day of birth (E19). Furthermore it remains closed 24 hours after birth (D1) but appears to be developing as evidenced by considerable sloughing of cells at it's internal free-surface. The sloughing continues but declines at the older ages as the canal's lumen increases in size resulting in a canal which is indistinguishable from that of an adult at D25. Thus, not only can the AOS's role in prenatal olfaction be discounted but it appears unlikely that it could be mediating chemosensation in the early postnatal period. Preliminary studies on the mouse fetus utilizing the 2-DG technique to compare the metabolic activity of the Main and Accessory Olfactory Bulb and their respective peripheral mucosae are discussed in relation to potential functional activity of these systems *in utero*.

[1] We thank Andrew Clancy and Tom Shoenfeld for help on this project. Supported by NINCDS grant 14453.

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Ultrastructural Characterization of Substance P Immunopositive Granule Cells in the Hamster Accessory Olfactory Bulbs. TAICHANG JANG (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545), RICHARD M. KREAM (Tufts University School of Medicine, Boston, MA 02111) and FOTEOS MACRIDES (Worcester Foundation for Experimental Biology).

A population of granule cells in the accessory olfactory bulbs (AOB) of adult male hamsters is immunopositive for the tachykinin substance P (SP). The present study examined the ultrastructural features of these neurons. Vibratome-sectioned olfactory bulbs were processed with anti-SP serum and a modification of the avidin-biotin complex method, followed by conventional electron microscopic procedures. The SP immunoreactivity was observed in the granule cell layer (GRL) and combined mitral body/external plexiform layer (MBEPL) of the AOB. The small SP immunoreactive neurons found in the GRL exhibited the typical appearance of granule cells. They possessed a relatively large nucleus with chromatin clumped at the periphery, a very thin rim of SP immunopositive cytoplasm, and immunopositive initial processes which extended into the GRL or superfically into the MBEPL. The immunopositive granule cells often formed a cluster with immediately adjoining granule cells which had similar ultrastuctural features but lacked SP immunopositivity. SP immunoreactive processes exhibiting pre- or postsynaptic features were distributed throughout the MBEPL. These processes appreared to be the distal dendrites of granule cells and to form typical dendrodendritic synapses with the mitral cells. The granule cells modulate output neurons in the olfactory bulbs and have generally been found to mediate inhibitory functions. SP, on the other hand, is widely accepted as an excitatory neurotansmitter in the central nervous system. Although the physiological function of SP in the olfactory system has not yet been established, the existence of SP-like immunoreactivity in otherwise apparantly conventional granule cells of the AOB brings into question the universality of the generalizations that SP is a depolarizing compound or that olfactory bulb granule cells are inhibitory interneurons.

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Accessory olfactory bulb neurons respond to liquid delivery of odorants to vomeronasal organ. JUN INOUCHI, JOHN L. KUBIE and MIMI HALPERN (SUNY Health Science Center at Brooklyn)

One of the major distinctions between the vomeronasal and main olfactory systems is the medium of odor delivery to the appropriate sensory epithelium. As we reported previously, when recording from the surface of the vomeronasal epithelium (VE), we observed responses to saturated vapor of n-amyl acetate (AA). These responses were similar to those recorded from the olfactory epithelium but 10 times smaller. In contrast, saturated vapor of earthworm wash (EWW) did not produce responses from the VE (Inouchi et al., Chemical Senses 13, 1988). In the present study we investigated the effects of liquid delivery of substances (prey washes, amino acids, standard odorants) applied to the exposed VE on single unit activity in the accessory olfactory bulb (AOB). EWW and goldfish wash (GFW), delivered as liquids to the VE, produced both excitatory and inhibitory responses in the AOB that were dose-dependent. These electrophysiological results correlate well with results of feeding studies that demonstrate the importance of the vomeronasal system in garter snake prey attack and prey trailing. Four nonvolatile amino acids were tested as liquids. Single unit firing of AOB neurons increased markedly to L-glutamic acid and L-arginine HCl, increased only moderately to L-alanine and was unaffected by L-proline. We used AA, D-limonene (LIM) and n-butanol (BUT) as standard odorants. The response to liquid AA was strong, and there was some increase in firing to LIM but none to BUT. These results demonstrate that the vomeronasal system is sensitive to a variety of odorants (natural and unnatural) and support the idea that under normal conditions liquid delivery of substances is an effective means of stimulating the vomeronasal system.

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Topographical Projection from Vomeronasal Organ to Accessory Olfactory Bulb. MARY WISGIRDA & MICHAEL MEREDITH (Florida State University, Tallahassee FL)

The vomeronasal organ (VNO) is an accessory chemosensory structure in all terrestrial vetebrates, except old world primates and birds, and has been shown to be involved in reproductive behaviors in many species, including the male hamster. Each organ is an elongated tubelike structure lying along the base of the nasal septum. Receptor cell axons form 3(+) nerve bundles which terminate in the accessory olfactory bulb, with the nerve from the anterior end of the organ being the most dorsal. Horseradish peroxidase was injected into a single nerve bundle in each of a series of male hamsters, to trace the connections from anterior, central and posterior sets of receptors into the accessory olfactory bulb. After a 48 hour survival period, animals were perfused and frozen sections of the olfactory bulbs and VNO processed using standard TMB histochemistry. Sections were examined in dark field for HRP reaction product (RP). The density of RP in 3 areas of each section was determined with computer-assisted image analysis. Anterior nerve injections labeled cells only in the anterior portion of the VNO. With posterior nerve injections, only the posterior end of the organ contained RP. Each nerve had labeled terminals throughout the glomerular layer of the AOB, but their distribution varied significantly. Injection of the most posterior (ventral) nerve produced a much greater density of RP in the ventral than in the dorsal half of the AOB with little variation medio-laterally. In contrast anterior nerve injections had more RP laterally than medially in the AOB and only a slight dorsoventral gradient. The results suggest a degree of topographic organization to this projection which will be investigated further.

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A Comparison of Detection Thresholds Determined by Charm Analysis, and Sensory Evaluation. A. B. MARIN, T. E. ACREE & J. BARNARD (Cornell University, Geneva, NY)

In a previous study, odor detection thresholds of standard aroma compounds were determined by a bioassay called charm analysis (Marin *et al., Chem. Senses*, 13, 435-438, 1988). Thresholds for some compounds varied among subjects while others did not. In the present study, thresholds for nine individuals from 16-19 years of age were determined using both charm analysis and a semi-ascending paired difference test (Lundahl *et al., J Sensory Studies*, 1, 291-306,1986). Odor detection thresholds of six standard aroma compounds determined by charm analysis, averaged over subjects in this study, were the same as those reported in the previous study. The charm threshold for one of these compounds,1,8-cineole, was related to the smell and taste thresholds for 1,8-cineole added to Concord grape juice. There was no difference found between odor and taste thresholds for cineole in the grape juice. However, the group threshold for pure cineole as determined by charm analysis at 95% confidence was (7-22) ng/stimulus or (7-22) μg/ml of extract while the group threshold for cineole in grape juice was (112-334) μg/ml. Therefore, charm analysis detects stimuli when they are above threshold as determined by sensory difference tests, and supports the use of charm analysis to study odor-relevant compounds in natural products.

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Concerning the Variability of Odor Thresholds. JOSEPH C. STEVENS, WILLIAM S. CAIN, and RANDALL J. BURKE (John B. Pierce Foundation Laboratory and Yale University, New Haven, CT 06519).

It is well known from earlier studies both in our own and several other laboratories that olfactory thresholds appear to be extremely variable across subjects. Subjects may differ by several orders of magnitude on the same compound and under the same procedure. The present study demonstrated that the greatest source of this variability is the fluctuation of any individual's threshold over time. By a forced-choice, ascending limits procedure each of three subjects gave 60 thresholds, two on each of 30 testing days and 20 each for three odorants: butanol, pyridine and beta-phenylethylmethylethylcarbinol (PEMEC). The variability of each subject was comparable in magnitude to that seen among large groups of subjects each tested only once. In contrast, the mean thresholds of the three subjects were similar in magnitude. Two lessons follow from this account. (1) It is unwise on the basis of a single threshold assessment to conclude that a given subject has especially good or poor olfactory sensitivity. On the other hand (2) the outcome also suggests that the extreme fluctuations of threshold from one test to another may tend to obscure real and stable average differences among subjects' olfactory keenness or dullness.

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Correlates of Hedonic Estimates in the Olfactory Evoked Potential. G. KOBAL, TH. HUMMEL, and E. PAULI (Department of Pharmacology and Toxikology, University Erlangen-Nuremberg, Universitätsstr. 22, D-8520 Erlangen, FRG)

The aim of the present study was to find correlates of stimulus induced emotions in the human olfactory evoked potential (OEP). 20 right-handed male volunteers participated in the experiments. During one experimental session either acetaldehyde in two concentrations, hydrogen sulphide or phenylethyl alcohol were presented to both nostrils in a randomized order. Subjects were instructed to indicate which nostril had been stimulated and - at the end of a sequence of 50 stimuli - the pleasant/unpleasantness of the odorants using a visual analogue scale. EEG was recorded from 14 positions (10/20 classification) referenced to linked earlobes. Acetaldehyde (low) and phenylethyl alcohol were rated as pleasant and acetaldehyde (high) and hydrogen sulphide as unpleasant. Only with acetaldehyde (high) stimuli the side of stimulation could be localized. The main differences in OEPs was that latencies of the component N1 increased with increasing pleasantness when the left nostril was stimulated ($p<0.05$). Acetaldehyde (low) stimuli, e.g., resulted in longer latencies (approximately 40 msec) than acetaldehyde (high) stimuli when the left nostril was stimulated. From our data we conclude that pleasant olfactory sensations are correlated with long latencies of the OEPs and unpleasant olfactory sensations with shorter latencies of the OEPs when the left nostril is stimulated. These results indicate that OEP recordings can be utilized to obtain quantifiable correlates of stimulus induced emotions.

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Quality Coding of Odorants for Normal Controls and Patient Populations. H.N. WRIGHT, Ph.D. and D.U. SMITH, Ph.D. (State University of New York Health Science Center at Syracuse)

Measurement of olfactory ability at our Olfactory Referral Center is accomplished by requiring observers to repeatedly identify ten different odorants in a closed set response mode. The results are cast into an Odorant Confusion Matrix (OCM) in which the rows represent the odorants presented, and the columns represent the responses. Quality coding is measured not by the percent correct identification as calculated from the main diagonal of the OCM, but rather by the distribution of the remaining responses not on the main diagonal. Patterns of off-diagonal responses were evaluated with multidimensional scaling techniques for normal controls (male vs. female) and different patient groups (viral, trauma, mucosal, aging, and congenital), and pseudohypoparathyroid patients (Gs unit normal, Gs unit deficient).

It will be shown that the quality coding of odorants is completely disassociated from percent correct identification. Further, patient groups distinguish themselves from one another on the basis quality coding as derived from the off-diagonal responses of OCM.

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CYTOSKELETON AND G-PROTEIN INTERACTIONS OF THE N-FORMYL PEPTIDE RECEPTOR

A.J. Jesaitis, J.O. Tolley, G.M. Bokoch and R.A. Allen. Research Institute of the Scripps Clinic, La Jolla, CA.

The N-formyl chemotactic receptor was identified in cytoskeletal, membrane, and detergent extracts of human neutrophils using a photoaffinity analog of f-Met-Leu-Phe-Lys (FMLPL-SASD-^{125}I). Molecular complexes of receptor and other proteins including G-proteins were also identified. Plasma membrane domains of cavitated human neutrophils were isolated on sucrose density gradients. The heavy fraction (ρ=1.16 g/cc) was enriched in the cytoskeletal proteins actin and fodrin. The light fraction (ρ=1.13 g/cc) was enriched in alkaline phosphatase activity, lectin binding and surface iodination sites, and G-proteins. Unoccupied N-formyl chemotactic receptors were found in the light fraction in unstimulated cells. Occupied receptors were moved from the light to the heavy fraction as cells were desensitized by prior exposure to chemoattractant at temperatures (15°C) that are not permissive for endocytosis, secretion, or superoxide production. Occupied, photoaffinity-labeled receptors, solubilized from the heavy fraction in octyl glucoside, sedimented like 4S particles at 4°C, while receptors from the light fraction sedimented like 7S particles. Inclusion of GTP in the detergent extraction cocktail converted all of the 7S receptor form to the 4S form (ED_{50}=10 nM). The nucleotide had no effect on the the sedimentation of the 4S form. The 7S form could be reconstituted with receptor derived from desensitized or fully responsive cells using detergent extracted light membrane fraction. Since desensitization is correlated to formation of receptor cytoskeletal complexes in the heavy domain of the plasma membrane, we infer that the resultant segregation of receptors from G-proteins in the plane of the membrane may regulate the interaction of receptors and G-proteins. This regulation may thus be a mechanism of control in chemotactic receptor-dependent responses.

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Conformational Dynamics of the Formyl Peptide Receptor. LARRY A. SKLAR (Scripps Clinic and Research Foundation, La Jolla CA 92037).

We have used real-time fluorescent methods and a family of fluorescent peptides containing 4, 5 and 6 amino acids to explore conformational dynamics and structural features of the formyl peptide receptor. Neutrophil activation proceeds via a rapid interconversion among three distinct receptor states - a slowly dissociating ternary complex of ligand, receptor, and G protein (LRG), a rapidly dissociating occupied receptor (LR), and a third, slowly dissociating complex "LRX" formed subsequent to, but not dependent on cell activation. Activation involves the binding of L to R at a diffusion limited rate, the formation of LRG essentially as rapidly as L binds to R but within a few hundred msec, and the activation of LRG at a rate proportional to the guanine nucleotide concentration (estimated to be ~ 10/sec). LRG forms homogeneously and there is sufficient G to accomodate at least 100,000 R per cell. Each activated receptor probably activates a small number of G. LRX forms (half-time 10 sec) and is internalized (half-time 3 min) without the assistance of microfilaments, possibly as a consequence of phosphorylation of LR. The three receptor states can be trapped on intact cells. LRG forms if L is added following depletion of high energy adenine and guanine nucleotides by glycolitic poisons. LR forms if cells are first ribosylated by pertussis toxin and depleted of energy prior to ligand. LRX predominates in untreated or ribosylated cells. While the peptides containing 4 and 5 amino acids are quenched upon binding to the receptor, the one containing 6 is not. In contrast, the carboxy terminal fluoresceins in the receptor-bound tetra- and pentapeptides are protected from antibody to fluorescein while the fluorescein is accessible to the antibody in the hexapeptide. Taken together these data suggest that the binding pocket of the receptor accomodates 5 and probably no more than 6 amino acid residues counting from the amino terminus.

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Phospholipid activated protein kinase C and calcium in signal transduction. H.M. Korchak, M.W. Rossi, W.A. Phillips, R.B. Johnston and T. Fujiki (U. of Pennsylvania)

Activation of neutrophils with ligands such as the chemotactic peptide f-Met-Leu-Phe triggers O_2^- generation and degranulation. Signalling is initiated by cleavage of phosphatidyl inositol 4,5 bisphosphate (PIP_2) to yield intra cellular signals inositol 1,4,5 trisphosphate (IP_3) and diacyl glycerol (DG) which trigger mobilization of Ca^{2+} and activation of protein kinase C (PKC). Addition of 10^{-7}M f-Met-Leu-Phe to neutrophils triggered rapid breakdown of PIP_2 and an increase in DG. PKC has been proposed as a mediator in multiple neutrophil functions, including O_2^- production. Phorbol myristate acetate (PMA), an activator of protein kinase C can bypass ligand-receptor interaction and activate O_2^- generation. In addition to generation of the cofactors Ca^{2+} and DG, activation of PKC is thought to involve translocation of PKC from cytosol to membrane. Neutrophil cytoplasts (vesicles of cytoplasm enclosed by plasmalemma) produce O_2^- in response to stimulants such as fMLP and PMA and are therefore a model system in which to study translocation. Activation of cytoplasts with 1 µg/ml PMA (5 min) or disruption in elevated Ca^{2+} (500 nM) elicited translocation of PKC activity (DG/PS/Ca-dependent histone phosphorylation) from cytosol to particulate fraction. Polyclonal, antipeptide antibodies recognizing consensus as well as α, β,and γ isozyme epitopes (Makowske et al, JBC 263:3402), were used to study PKC distribution. PKC immunoreactivity was observed in cytosol and membrane fractions of resting cytoplasts with antibody to consensus peptide; immunoreactivity was less in pellet than in cytosol. Addition of Ca^{2+} during cytoplast disruption or activation of cytoplasts with PMA, elicited increased immunoreactive 80 kDa species in the membrane fraction and less in cytosol. Antibody to β-PKC immunoblotted predominantly to an 80 kDa cytosolic polypeptide; this cytosolic immunoreactivity was decreased by activation with PMA or by disruption in Ca^{2+}, whereas no differences in the blotting patterns to antibodies to α-PKC or γ-PKC was observed. Therefore signalling may involve elevation of Ca, DG and translocation of the β isozyme.

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Lessons from the Leukocyte Story JUDITH VAN HOUTEN (University of Vermont, Burlington, VT 05405).

Every organism has sensory systems designed to extract information from its environment and to transduce this information into a useful form that causes the organism to respond. When the external information is a chemical or a mixture of chemical cues, the sensory process is referred to as chemoreception and the result can range from the sensations of taste and smell, familiar to us all, to the attraction of motile bacteria. While these chemoreception systems are wide-ranging, they do have aspects in common. They all appear to initiate at the membrane surface of a receptor cell by the interaction of a stimulus with a receptor molecule or, perhaps in some cases, with the membrane itself. This interaction is then transduced into an internal signal that is useful to the cell in the generation of action potentials or the release of neurotransmitter. In the case of multicellular organisms, these events result in a message sent to the central nervous system about the quantity and quality of the odor or taste stimulus.

Leukocytes are exposed to a more constant chemical environment compared to the *external* chemoreception systems serving taste, smell, and chemoresponse of unicellular organisms. Nonetheless, because receptor-initiated responses of the leukocyte are wide-ranging and the molecular mechanisms are now known in such detail (as the preceding talks will illustrate), there are lessons here for the chemical senses. The parallels and general themes in common with sensory transduction in the chemical senses systems ranging from Paramecium to vertebrates will be discussed. These themes will include: stimulus-receptor interactions; roles of G proteins; the second messengers calcium cyclic nucleotides, phosphoinositols, arachidonic acid; receptor down regulation and desensitiziation; membrane potential controls.

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Electron-Microscopic Demonstration of Olfactory-Marker Protein with Protein G-Gold in Freeze-Substituted, Lowicryl K11M-Embedded Rat Olfactory-Receptor Cells, Bert Ph. M. Menco (Department of Neurobiology and Physiology, O. T. Hogan Hall, Northwestern University, Evanston, IL 60208-3520, USA.)

Electron-microscopic immunocytochemistry was used to localize olfactory marker protein in olfactory epithelia. Rat olfactory-epithelial samples were rapidly-frozen, freeze-substituted with acetone, embedded at low-temperatures with Lowicryl K11M and labelled on the sections with polyclonal antibodies raised in goat against rat olfactory-marker protein and protein G-colloidal gold. Apart from the aforementioned use of acetone, substitution was carried out in the complete absence of chemical fixation, i.e, neither aldehydes nor OsO_4 were used. This procedure resulted in localization concurrent with a good ultrastructural preservation. Olfactory-marker protein was present throughout the cytoplasmic compartments of dendrites and dendritic endings of olfactory-receptor cells, but it was not found in organelles such as mitochondria. Olfactory-marker protein was found only in dendritic endings of olfactory-receptor cells mature enough to have given rise to cilia, but these cilia displayed less labelling than dendrites and dendritic endings. Olfactory-marker protein was not found in apices and microvilli of neighboring olfactory-supporting cells. *This work was supported by grants from NSF (BNS-809839) and the Erna and Victor Hasselblad Foundation.*

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Stimulant and Diffusion Pathways in Gustatory Sensilla on the Galeae of Four Species of Lepidopteran Larvae. VONNIE D. SHIELDS and R.Y. ZACHARUK (Biology Dept., University of Regina, Regina, Sask., Canada. S4S 0A2).

The galeae of fifth instar larvae of bertha armyworm, Mamestra configurata, tobacco hornworm, Manduca sexta, gypsy moth, Lymantria dispar, and fall armyworm, Spodoptera frugiperda, bear six sensilla. Of these, only two styloconic pegs are permeable to cobalt ions into the terminal dendritic channel. In three species, mercury ions seem to be trapped only in the terminal pore area, and in all species, lead ions did not appear to enter the pore system of these pegs. The ion loading technique will be described and the results shown, as viewed by light microscopy. The fine structure of the terminal pores will be compared by SEM and TEM for the four species and discussed in relation to their loading with the three cations used.

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Divalent Cation Loading and Effects in Gustatory Sensilla of the Tobacco Hornworm by LM, STEM, EDX Probe and Electrophysiology. R.Y. ZACHARUK (Biology Dept., University of Regina, Regina, Sask., Canada. S4S 0A2 and J.L. FRAZIER (Agricultural Products Department, E.I. DuPont de Nemours and Co., Wilmington, DE. 19898)

The two styloconic taste pegs on the galea of fifth instar tobacco hornworms, Manduca sexta L., were used in this study. Divalent cations (Co^{++}, Hg^{++}, La^{++}, Pb^{++}) were applied by soak, and by micropipette electrode during tip stimulation and simultaneous single cell electrophysiological recording of response. Immediately following treatment, the cations were precipitated with sulfide and processed for light microscopy (LM) and for electron microscopy by TEM and EDX probe analysis by STEM. Some correlates of fine structure, cation permeation, and changes in response to stimulation will be discussed.

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Immunohistochemical Localization of Components of the Secretory Immune System in the Olfactory Mucosa. MARILYN L. GETCHELL, KENNETH L. GWINN* & THOMAS V. GETCHELL, Depts. of Anatomy & Cell Biology and *Otolaryngology, Wayne State University School of Medicine, Detroit, MI 48201.

The localization of four components of the secretory immune system, as well as IgG, lactoferrin and lysozyme, in the olfactory mucosae of rats and salamanders was investigated using direct and indirect immunofluorescence techniques. The major component of the secretory immune system is secretory IgA in mammals and secretory IgM in amphibians; this complex contains polymeric immunoglobulin molecules joined by J chain, both of which are synthesized by local plasma cells, and secretory piece, which is synthesized in glands and translocates the complex across gland cell membranes. Bowman's glands (BG) in both species were immunoreactive (ir) following staining with an antibody to secretory IgA, which recognizes IgA, J chain and secretory piece; an antibody to IgA resulted in immunoreactivity in rat BG only. BG were also ir for secretory piece and J chain in both species and for IgM in the rat. Both acinar and duct cells were ir for various components of the secretory immunoglobulin complex. BG in the rat also were ir for lactoferrin and lysozyme; salamander BG demonstrated immunoreactivity for lysozyme only. Plasma cells in or near BG acini in the lamina propria of both species were ir for secretory IgA and IgM, and for IgA in the rat only. IgG immunoreactivity was found in plasma cells in both species and generally throughout the connective tissue of the lamina propria of the rat. Blood cells resembling polymorphonuclear leukocytes were ir for lactoferrin in the salamander lamina propria. Thus, the glands and plasma cells of olfactory mucosa produce components of the secretory immune system as well as non-specific immunological factors that confer protection against both local infections and invasion of the central nervous system by infectious agents via this port of entry.

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Evidence for Topography in the Olfactory Bulb of the Frog. H.J. DUNCAN, W.T. NICKELL, M.T. SHIPLEY and R.C. GESTELAND (Department of Anatomy & Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267).

We have presented evidence that discrete portions of the olfactory epithelium (OE) of the frog *Rana pipiens* project to discrete regions of the glomerular layer of the olfactory bulb (AChemS X). This preliminary finding has been substantiated and extended. Iontophoretic injections of WGA-HRP were made in the *eminentia olfactoria* of the ventral OE or in the dorsal OE. Transport of label (visualized as the TMB reaction product) to the bulb was seen after as few as two days, and label remained in the bulb after as many as 21 days post-injection. With relatively constant iontophoretic injection parameters, injection site size varied considerably and appears to correlate with the density and extent of labelling in the glomerular layer. With very large injections, WGA-HRP was frequently taken up in the dorsal and ventral epithelia, and labelled glomeruli tended to be evenly distributed in the bulb. The addition of atropine to the anesthetizing solution before injection of WGA-HRP suppressed mucous secretion and reduced diffusion of the label to the opposite epithelium.

In cases where the injection site was successfully restricted to a portion of the *eminentia olfactoria*, a very clear pattern of labelling was revealed in the bulb, indicating that fibers from this region terminate more densely in the lateral portions of the bulb, although light labelling was found in all regions of the bulb. In marked contrast, injection sites restricted to the dorsal epithelium resulted in greater labelling in the medial portions of the bulb.

In summary, we have found that projections from the *eminentia olfactoria* of the ventral OE terminate preferentially in the lateral region of the olfactory bulb, and projections from the dorsal epithelium terminate in the medial bulb. The injection sites and patterns of label in the olfactory nerve are being analyzed further to search for additonal topography within these two gradients. In addition, small injections of retrograde markers into the lateral or medial regions of the bulb will be attempted to verify the dorsal-ventral origins of projecting neurons.

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Novel Synapses Associated with Light Cells in Rabbit Fungiform Taste Buds. JOHN C. KINNAMON, SUZANNE M. ROYER AND ANDREW BARBER (University of Colorado at Boulder and the Rocky Mountain Taste and Smell Center, Denver)

We have previously reported ultrastructural differences between synapses in fungiform taste buds and in the taste buds of foliate and circumvallate papillae of the mouse (Kinnamon *et al.*, AChemS 1988). Recently we have studied synaptic connections in foliate taste buds of the rabbit (Royer & Kinnamon, AChemS 1988). Now we have begun to examine rabbit fungiform taste buds. In general, our observations are similar to those of Murray (1973), who noted several differences between fungiform taste buds and those of the foliate papillae of the rabbit. Specifically, light and dark cells in fungiform taste buds are more similar in morphology contrasted with foliate taste buds, where light and dark cells are quite distinctive. Murray also reported that synapses in rabbit fungiform taste buds were associated only with type III cells, similar to his and our observations on foliate taste buds in rabbits. In contrast, we have obtained evidence for synaptic contacts from light cells onto nerve fibers in fungiform taste buds of the rabbit. These synapses are characterized by very large aggregations of clear vesicles in the synaptic region. Such synapses resemble those observed by Kinnamon *et al.* (AChemS 1988) in the fungiform taste buds of the mouse. We have previously demonstrated that mouse fungiform synapses are fewer but much more elaborately developed than either foliate or circumvallate synapses in the mouse. Hence, in both the rabbit and the mouse, it appears that fungiform synapses may be more complex than synapses in other lingual papillae. The functional significance of these elaborate synapses in fungiform taste buds is not known. We speculate, however, that fungiform synapses may be much more active than synapses in either foliate or circumvallate taste buds. In addition, we believe that our observation of synapses from light cells onto nerve process in rabbit fungiform taste buds is the first demonstration that not all synapses in rabbit taste buds are associated with type III cells.

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The Ultrastructure of Nerve Terminals in the Apical Region of the Frog Olfactory Epithelium. B. ZIELINSKI, M.L. GETCHELL AND T.V. GETCHELL (Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201).

Previous studies have demonstrated that the olfactory mucosa of the leopard frog contains numerous intraepithelial substance P-immunoreactive fibers that branch and run parallel to the olfactory epithelial surface for short distances. This orientation optimized the chances of locating terminals of these fibers for ultrastructural study. Electron microscopic investigation revealed nerve terminals in the apical olfactory epithelium just proximal to the zonula adherens between adjacent sustentacular cells, and, less frequently, between sustentacular cells and olfactory receptor neurons. The varicosities were located about 20 nm from the membranes of adjacent cells and contained numerous vesicles and mitochondria. Two types of vesicles comingled within each terminal: large vesicles with a mean diameter of 104 ± 18 nm (N=80) that contained dense cores and small vesicles with a mean diameter of 60 ± 14 nm (N=124) that lacked dense cores. The terminals appeared to contain a greater number of large dense-cored vesicles than small clear ones. Synaptic membrane specializations were not observed between the terminals and adjacent cells. Although the contents of the vesicles were not identified immunocytochemically, large dense cored vesicles within nerve terminals in other tissues have been shown to contain neuropeptides such as substance P and VIP while small clear vesicles have been shown to contain acetylcholine, serotonin or enkephalins. These presumed peptidergic nerve terminals located near the olfactory epithelial surface most likely represent the sensory free nerve endings of the trigeminal nerve.

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Transport Properties and Proteins in Canine Circumvallate Papillae. S.A. SIMON and V.F. HOLLAND (Dept. of Neurobiology, Duke Univ. Durham, N.C.); D.J. BENOS (Dept. of Physiology and Biophysics, U. of Alabama at Birmingham, Birmingham, Ala., and G.A. ZAMPIGHI (Dept. of Anatomy, U.C.L.A. Los Angeles, Ca.) *

The open circuit potential, Voc, and the short circuit current, Isc, responses of isolated circumvallate papillae (CP) from canine lingual epithelium to NaCl were measured in a Ussing chamber. Both Voc and Isc monotonically increased until they saturated at about 0.4 M NaCl. The responses of Isc were approximately the same magnitude at 0.4 M NaCl (~60 uA/cm2) as the responses of an equal area of canine epithelium containing fungiform papillae (FP) whereas the responses of Voc at 0.4 M NaCl (~10 mV) was about half that of found in FP. Such data are of interest in understanding the spatial responses of lingual epithelium to NaCl because CP have a much greater number of taste cells per unit area than FP. With 0.4 M NaCl in the mucosal solution, 0.1 mM amiloride added to the mucosal solution or 1 mM ouabain added to the serosal solution inhibited the Isc.

Proteins involved in ion transport were identified in taste buds in canine circumvallate papillae using immunocytochemistry and histochemistry at the optical level. Polyclonal primary antibodies raised against amiloride inhibitable sodium channels from bovine kidney cells were visualized with a secondary anti- rabbit antibody labeled with fluorescein. The fluorescent probe was primarily localized in taste buds and on cells surrounding them at the region of the taste pore. The Na-K-ATPase and Ca-ATPase were primarily confined to taste buds but were also identified in epithelial cells.

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The Effect of Neonatal Capsaicin Administration on Trigeminal Nerve Fibers in the Nasal Cavity. W.L. SILVER, L.G. FARLEY, WOMBLE, M.* and T.E. FINGER*. Wake Forest Univ., Winston-Salem, N.C. and *Univ. of Colorado Medical Center, Denver, CO.

Chemical stimuli entering the nasal cavity may stimulate at least two types of chemoreceptors in the nasal mucosa: those of the olfactory system and those of the trigeminal system. Evidence is accumulating which suggests that nasal trigeminal chemoreceptors, usually associated with painful or irritating chemical stimuli, are a class of pain receptors rather than a separate chemical sense. These pain receptors appear to belong to the class of neurons referred to as "capsaicin-sensitive" sensory neurons. Capsaicin, the active ingredient in chili peppers, selectively blocks primary nociceptive neurons such that they are incapable of generating or transmitting impulses. Systemic capsaicin treatment in both adult and neonatal animals produces an insensitivity to noxious chemical stimuli, apparently due to the depletion of substance P, or other neuropeptides, from nociceptive fibers. Previously, we showed that chronic capsaicin administration in adult rats eliminates or severely reduces trigeminal nerve responses to chemical stimuli (Silver et al., 1985). In the present experiments we examine the effects of neonatal capsaicin administration on trigeminal nerve fibers in the nasal cavity. A 1% capsaicin solution or the vehicle (control: Tween 80, ethanol, and saline) was injected into 2 day old rat pups (50 mg/kg). After 40 days of age the ethmoid nerves of all the control animals and of 25% of the experimental animals responded responded normally to the stimuli tested (amyl acetate, cyclohexanone, propionic acid). However ≈ 75% of the experimental animals failed to respond to any concentration of these three compounds while responses to mechanical stimulation were unaffected. After the recording sessions, rats were perfused with 4% buffered paraformaldehyde and their nasal epithelia reacted immunocytochemically for substance P (SP) and calcitonin-gene-related-peptide (CGRP). In controls, intraepithelial fibers which were immunoreactive for both SP and CGRP were seen, a few of which reached the surface of the epithelium. Immunoreactive fibers also were seen in olfactory nerve fascicles beneath the epithelium. The same pattern was seen the capsaicin treated rats which responded normally to the chemical stimuli. In capsaicin-treated rats which did not respond to chemical stimuli, however, no intraepithelial immunoreactive fibers were seen, while immunoreactive fibers in olfactory nerve fascicles were sparse. These results suggest that trigeminal nerve fibers in the nasal cavity which respond to irritants may be polymodal nociceptors which contain SP.

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Taste Mixtures can lead to Synthesis of new "Non-Basic" Tastes, or Suppression. ROBERT P. ERICKSON, ZOE S. WARWICK, SUSAN S. SCHIFFMAN (Departments of Neurobiology, Psychology and Psychiatry, Duke University)

When two basic tastes or other unitary-tasting stimuli are combined, the perception of this mixture may also appear unitary (as one taste) (Physiol. Beh. 25, 1980, 527-533; 28, 1982, 57-62). This poses a problem for the "four tastes" position in that it suggests the existence of tastes between and in addition to the basic four. In the labeled-line view, this can be accounted for by suppression (and thus disappearance) of one of the tastes by the other stimulus; then the mixture should be the same as (indistinguishable from) the suppressor stimulus, and thus still different (discriminable) from the suppressed stimulus. In the across-fiber pattern view, a new singular taste discriminable from either component may be formed. An analogy from vision is the following: a blue-green formed from the mixture of blue and green is singular but distinguishable from either component. If discriminability of a unitary mixture from both of its unitary components occurred in taste as in color vision, then more than the four basic sensations would be indicated. In the present study the discriminability of taste mixtures previously shown to be unitary was tested. Intensities of the components were equated with the intensity of the mixture. With NaCl and NH_4Cl, the mixture was not highly distinguishable from NaCl, but was clearly distinguishable from NH_4Cl, suggesting some suppression of the taste of NH_4Cl by NaCl. Other mixtures (HCl/NaCl, $MgCl_2$/NaCl, HCl/Q_2SO_4) were clearly distinguishable from each component suggesting synthesis of new singular tastes. (Sugars tend to not form singular mixtures with these stimuli). These data support the position that while suppression may operate in some cases, tastes other than the "basic four" exist as well. Such intermediate unitary tastes, reduced in similarity to (suppressed in comparison with) the components, may previously have been interpreted as "suppression."

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Bitter Side-tastes and Synergism in Mixtures of Sweeteners. SARA J.S. MIZE & ROBERT A. FRANK (University of Cincinnati).

As recently reported by Frank et al. (1988, Chem. Senses, 13, 689), mixtures of some sweeteners exhibit synergism while others do not. Since many of the more pronounced synergistic effects were observed in bitter-sweet/purely sweet substance mixtures, several experiments were performed to assess the role of bitter side-tastes in synergism. In the first study, taste quality profiles were collected for three concentrations of nine sweeteners (acesulfame K, aspartame, cyclamate, fructose, glucose, sucrose, stevioside, saccharin, xylitol). These data were used to select two bitter-sweet substances (acesulfame, saccharin) and a purely sweet substance (aspartame) for further study. In the next experiment, subjects made sweetness and bitterness ratings for three concentrations of acesulfame, aspartame and saccharin plus mixtures of acesulfame/aspartame and acesulfame/saccharin. In the bitter-sweet/purely sweet substance mixture i.e., acesulfame/aspartame, the bitterness of acesulfame was significantly reduced in the mixture. However, bitterness was not reduced in the acesulfame/saccharin mixture, a mixture of two bitter-sweet substances. In the final experiment, subjects judged the sweetness of acesulfame/aspartame and acesulfame/saccharin mixtures using a factorial mixture design. To assess mixture interactions, "self-mixture" data were also collected (see Frank et al., 1988). It was found that acesulfame/aspartame mixtures showed pronounced synergistic effects, whereas acesulfame/saccharin mixtures showed no synergism. This pattern of results suggests that bitter suppression in mixtures may play some role in synergistic effects.

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Synergism in Mixtures of Sweetners: Convergent Validation from Equal Volume Mixture Experiments. ROBERT A. FRANK & SARA J. S. MIZE (University of Cincinnati).

In a recent experiment that assessed the sweetness of binary mixtures of eight sweeteners, it was found that some of the mixtures exhibited syenrgism, i.e. the sweetness of the mixtures exceeded what would be expected if the component sweeteners had been mixed with themselves. Convergent validation for the synergistic effects was sought in a second set of experiments involving equal volume mixtures. In this experiment, equal volume mixtures of acesulfame/aspartame, aspartame/saccharin, acesulfame/xylitol, aspartame/stevioside, sucrose/cyclamate, glucose/cyclamate, sucrose/fructose, fructose/xylitol, fructose/glucose and fructose/stevioside were studied. It was hypothesized that mixing equal volumes of sweetness-intensity matched solutions would produce mixtures of the same sweetness as the component solutions. Deviations from equal sweetness would represent examples of synergism or suppression. The component solutions were chosen to match the sweetness of 0.25 sucrose. Mixtures were prepared by combining equal volumes of the component solutions. Twenty subjects made difference judgments for pairs of stimuli, rating whether the mixtures where greater than, less than or equal to the sweetness of the component solutions. The data were analyzed by calculating the mean difference between the mixture and component ratings for each binary mixture. It was found that the sweetness of the mixtures exceeded the sweetness of the components for some mixtures, but not others. The greatest increase in mixture sweetness was observed for the acesulfame/aspartame mixture while the fructose/glucose mixture was about as sweet as its components. The pattern of results in the present study and our previous mixture research were in good agreement. Therefore, the equal volume mixture data provide convergent validation for the phenomenon of synergism and the pattern of results observed in the original mixture research.

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Odor Mixtures: Does Complexity Enhance Masking? R. BLAIR SKINNER and WILLIAM S. CAIN (John B. Pierce Foundation Laboratory and Yale University, New Haven CT 06519)

Existing data imply that as mixtures become more complex their perceived intensity departs more and more from simple perceptual additivity. Any given component in a mixture should therefore seem weaker as complexity increases. We tested this hypothesis in an experiment where 12 subjects rated both the total perceived intensity of vapor-phase mixtures of up to four iso-intense components and the perceived intensity of target odors embedded in the mixtures. The data supported the hypothesis that complexity did indeed increase the suppression or masking of the target odors. Nevertheless, gas chromatographic measurements revealed that much of the effect lay in suppression of vapor-phase concentration. As mixtures became more complex, the vapor pressure of their components progressively decreased. The use of target odorants suitably amplified to compensate for physical suppression eliminated the psychophysical suppression as well. The results lead us to suspect that various phenomena seen in vapor-phase mixtures may have their origin in departures from common assumptions about physical additivity.

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A Comparison of Response Characteristics of Airflow and Pressure Transducers Commonly Used in Rhinomanometry. RICHARD E. FRYE (Sensonics, Inc., Haddonfield, NJ) and RICHARD L. DOTY (Smell and Taste Center, and Department of Otorhinolaryngology and Human Communication, School of Medicine, University of Pennsylvania, Philadelphia, PA).

Although the ranges in which pneumotachometers evidence linear output to static flows are available from literature reports, this is not true for measures of output reliability within these ranges, or for measures of the stability of output functions resulting from the input of dynamic non-sinusoidal flows (such as occur during nasal breathing). Furthermore, the influence of the type of requisite pressure transducer used in conjunction with the pneumotachometer on such measures is not known. To provide information on these points, we determined the dynamic and static responsiveness of three pneumotachometers commonly used in rhinomanometry, in combination with three requisite pressure transducers. In general, (a) output reliability depended on the pneumotachometer/pressure transducer combination and was not readily predictable from the reliabilities of the individual components, (b) heating influenced pneumotachometer reliability, and (c) differences in accuracy existed among transducer combinations at high, but not low, flow frequencies. The findings suggested that a single calibration factor, as supplied by most pneumotachometer manufacturers, is inadequate for accurately measuring the full range of flows produced in sniffing and breathing tasks and that the measurement of complex wave forms, even within the dominant low frequency range of breathing, requires pneumotachometers which accurately respond to relatively high frequencies.

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Color-induced odor enhancements behave like small intensity increments. G.S. SHAFFER, J.E. BECK, and D.A. ZELLNER (Department of Psychology, Shippensburg University, Shippensburg, PA 17257)

Prior research has found that adding coloring to odor solutions increases perception of odor intensity for some solutions but not others. If adding coloring is equivalent to adding a constant amount of odorant then the Weber-Fechner Law predicts a more detectable odor enhancement when coloring is added to weak odor solutions than to strong ones. Three groups of subjects (24 Ss each) rated the intensity of lemon and orange extract in distilled water (Group 1=0.83%, Group 2=3.33%, Group 3=13.33%). One instance of the lemon and orange solutions were clear for each group and the other instance was colored with food coloring (yellow for lemon and orange for orange). The lemon odors were significantly enhanced by the coloring only at the lowest concentration [t(23)=5.02, p(.001] but not at the higher ones [t(23)=1.13, p).05; t(23)=1.55, p).05]. The orange odors were significantly enhanced by the color at all three concentrations, but more at the lower concentration [t(23)=2.92, p(.01] than at the higher ones [t(23)=2.20, p(.05; t(23)=2.58, p(.05]. The results support the idea that adding coloring to odor solutions increases the perception of odor intensity by a small constant factor which is more easily noticed at lower base intensities.

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Colors elicit olfactory percepts. D.A. ZELLNER, J.A. KAJIC (Department of Psychology, Shippensburg University, Shippensburg, PA 17257) and M.A. Kautz (Department of Psychology, The American University, Washington, D.C. 20016)

Odor intensity ratings are higher for colored than for clear solutions. Is this due to a response bias or a perceptual change? 40 subjects were recruited for an "odor discrimination study". Each subject compared a standard odorant (1.25% strawberry fragrance in distilled water) with eight comparison stimuli (0, 0.31, 0.62, 0.94, 1.25, 1.56, 1.88, or 2.19% strawberry fragrance). Red food coloring was added to the standard stimulus (Group 1) or the comparison stimuli (Group 2). Subjects were told that the odorless red food coloring served to distinguish the standard from the comparison stimuli and should not influence their ratings. Subjects sniffed the standard followed by a comparison bottle and reported whether the comparison was stronger or weaker than the standard. In post-experimental inquiry, subjects declared that color had not influenced their responses. The central finding is that subjects rated the 1.25% comparison stronger than the standard (also 1.25%) more often when the comparison was red and the standard was clear (Group 2) than when the comparison was clear and the standard was red (Group 1) (p(.05). This suggests that coloring a solution truly makes it smell stronger.

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Parosmia among Patients with Olfactory Complaints. JAMES W. DONNELLY, WILLIAM S. CAIN, and APRIL SCOTT (Connecticut Chemosensory Clinical Research Center, University of Connecticut Health Center, Farmington, CT 06032)

Parosmia, either in the form of phantom odors or distortions of quality, poses a vexing clinical problem. Among patients seen at the CCCRC, about one-quarter report a history of phantosmia alone, one-fifth distortions alone, and about five percent both. In general, the phantoms have an unpleasant quality variously described as foul, putrid, burnt, or rubbery. Distortions also have an unpleasant character; such odors as coffee and peanut butter often smell rancid. Of patients with phantosmia, about two-thirds have experienced their phantoms at least a few times a month, with almost half reporting daily or almost daily occurrences. Furthermore, it matters little whether or not the patient has actual smell functioning; surprisingly, phantosmia occurs at least as frequently among anosmics as hyposmics. Hence, the problem often has annoying and even psychologically disruptive proportions. Does phantosmia associate more with one major etiological category than another? The category of head trauma displayed the highest frequency, with about four out of ten patients affected. The categories nasal/sinus disease and post-URI each displayed an occurrence of about one-quarter. The distribution differed for patients with distortions. Most notably, distortions occurred about three times more frequently among post-URI patients than among nasal/sinus disease patients. This disparity seems to reflect, however, the frequency of hyposmia among patients with distortion. Post-URI patients have about three times the frequency of measurable functioning and hence three times the opportunity to experience distortions.

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Olfactory Sensitivity, Nasal Resistance, and Autonomic Function in Patients with Multiple Chemical Sensitivities. DANIEL A. DEEMS, RICHARD L. DOTY, RICHARD E. FRYE, ROBERT PELBERG, and AARON SHAPIRO (Smell and Taste Center, Department of Otorhinolaryngology and Human Communication, and Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, PA)

A frequent, if not predominant, complaint of persons reporting symptoms of multiple chemical sensitivities (MCS) is that of heightened sensitivity to smells. In this study odor detection thresholds for phenyl ethyl alcohol (a major component of rose oil) and methyl ethyl ketone (a common solvent) were measured in 18 persons exhibiting symptoms of MCS and in 18 matched normal controls. In addition, nasal resistance, blood pressure, heart rate, and respiration rate were determined before and after the olfactory tests. Scores on the Beck Depression Inventory (BDI) were obtained prior to testing. Although olfactory thresholds were equivalent in the two study groups, the MCS group evidenced significantly higher nasal resistances, respiration rates, and BDI scores. Decreases in systolic blood pressure and pulse were noted in both groups across the test sessions. These results do not support the hypothesis that MCS is associated with greater olfactory threshold sensitivity (at least to the two target chemicals), but is associated with depression, increased respiration rate, and decreased nasal airway patency.

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Diet and the Preference for Fat in Selected Foods. D.J.MELA (Monell Chemical Senses Center)

The relationship between hedonic responses to fats in foods and their dietary acceptance and actual consumption are unclear. Previous studies have typically employed only dairy products as sensory stimuli and used limited methods of dietary assessment. Sixteen subjects kept detailed dietary records for 10 days, and then took part in sensory testing. Stimuli consisted of 10 different foods, prepared with 2 to 5 fat levels within each food type. Subjects rated each individual stimulus for pleasantness on a 9-point category scale. In addition, all of the fat levels of each stimulus were ranked for preference. Preliminary analyses show that there were wide individual variations in the most preferred fat level for each stimulus and no consistent relationships in ratings among the different test foods. The mean dietary fat intake was 38% of Kilocalories, and overall (all stimuli, subjects, and fat levels) optimal sensory preference was a 47% fat mix. Preferences for fats in the test stimuli were unrelated to dietary intakes and indices of body composition. These results suggest that documentation of individual sensory preferences for fats and evidence for relationships between measured preferences and actual dietary fat consumption may be highly dependent upon the specific test stimuli.

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Learned Flavor-calorie Conditioning Alters Food Intake in Man. BEVERLY J. TEPPER and RICHARD D. MATTES (Monell Chemical Senses Center).

The purpose of this study was to examine the role of sensory experience in determining food intake in free-living individuals. Sixteen, normal weight adults (9 men; 7 women) kept a daily diet record for 28 days. The study consisted of three phases; baseline, training and experimental. During baseline, no lunches were provided. During the 10-d training phase, subjects received on alternate days either a high-calorie (HC) lunch (macaroni and cheese, salad, bread, gelatin, soda) with one distinctive flavor or a low-calorie (LC) version of the same lunch with a different distinctive flavor. The experimental phase consisted of 2, 5-d blocks during which the flavors of the lunches were switched. Half the subjects received the LC lunch with the high-calorie flavor for 5 consecutive days followed by the HC lunch with the low-calorie flavor for 5 days. The remaining subjects received the meals in the opposite order. When fed the HC lunch with the low-calorie flavor, 25% of subjects (i.e., responders) initially increased their daily food intake relative to both baseline and the end of training in accordance with their acquired sensory experience. These same subjects initially decreased their intake of the LC lunch with the high-calorie flavor. Food intake gradually returned to baseline following both manipulations. The remaining subjects (i.e., non-responders) displayed no flavor-calorie conditioning but maintained consistent caloric intakes throughout the experiment. These findings suggest that at least for some individuals, flavor-calorie conditioning might provide an important signal for the maintenance of daily food intake.

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The Perceptual Similarity of Substituted Benzenes and Pyridines as a Function of Steric Hindrance

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Differences between the odor percepts of twenty 1,3-dialkylbenzenes and 2,6-dialkylpyridines are based mainly on two perceptual dimensions. The overall perceptual similarity of the benzene/pyridine pairs seems to depend on accessibility to the pyridine nitrogen atom and steric hindrance to the aromatic ring in a nonmonotonic way. However, a linear relationship between a unidimensional percept and steric hindrance exists. This conclusion is based on multidimensional scaling and odor profiling studies. This set of odorants may help to elucidate the receptor mechanisms underlying odor discrimination.

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Sex differences in responsiveness to odors in 6- and 9-month old infants.

Hilary J. Schmidt (Monell Chemical Senses Center & New Jersey Medical School)
Gary K. Beauchamp (Monell Chemical Senses Center)

In two experiments the responses of 9- (Exp 1) and 6-month-old (Exp 2) infants to odorized objects were explored. In each study 42 infants (21 males and 21 females) were familiarized in successive trials with two rattles which differed in both appearance and odor. The size and shape of the rattles were identical but they were different colors and patterns: one was odorized the other had no odor. The rattle that was odorized was counterbalanced across infants and for half the infants the odorized rattle was pleasant (methyl salicylate) and for the others it was unpleasant (butyric acid). Odors were matched for perceived intensity and approximately equated for trigeminal component. Four, 30 second, test trials followed the familiarization trials. On test trials, the two rattles were placed side by side on a table in front of the infant. Infants were allowed to freely select between and explore the objects during each trial. The left-right positions of the objects were switched between test trials in an ABBA design. Infants reactions were videotaped throughout. The amount of time that the infant spent exploring each object during test trails was analyzed from the videotapes.

Analyses revealed that in both age groups female infants spent significantly more time exploring the odorized rattle than the non-odorized rattle during the test trials, while male infants did not. Males in contrast spent about equal time exploring each rattle. Response patterns suggested that this effect was mediated by the odor and not due to sex differences in general responsiveness. This is an important result which suggests that the documented adult female superiority in odor tasks may be determined in part by genetic as opposed to experiential factors.

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Multidimensional Scaling of Perceived and Imagined Odors. BRIAN J. LYMAN (Monell Chemical Senses Center)

The topic off mental imagery has been an increasingly popular area of research in cognitive science. The issue of whether olfactory images (i.e., olfactory analogs of visual images) are represented in working memory in a like fashion as are olfactory percepts was examined in two experiments. In Experiment 1, 20 subjects judged the similarity of all possible pairs of 10 common, actual odors. In another session, they judged the similarity of all possible pairs of 10 odors that they were asked to imagine. In Experiment 2, 15 subjects replicated the two sessions of Experiment 1. In addition, subjects also spent two sessions making judgments about the similarity of actual odors to imagined odors and imagined odors to actual odors. Multidimensional Scaling (MDS) procedures were used to assess the correspondence between the stimulus spaces derived for actual and imagined odors. The results from Experiment 1 indicated that when the data for real and imagined odors were modeled in separate solutions each odor, whether imagined or perceived, tended to be portrayed in the same place in the derived stimulus spaces. The results from Experiment 2 showed that when all similarity judgments were modeled together in the same solution, imagined and perceived odors of the same referent tended to cluster together in the stimulus space. These data are interpreted in terms of a concept of "second order isomorphism". It is suggested that although imagined odors are not necessarily perceived in the same fashion as actual odors, they bear some structural similarity to actual odors. At the least, the relationship among members of a set of actual odors are preserved among members of a set of imagined odors.

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On the effect of gymnemic acid in a lesser ape, the gibbon.

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The gibbons (Hylobates lar; Fam. Hylobatidae) belong to the Hominoidea. This subfamily branched off from Cercopithecoidea about 24 million years ago. Molecular myoglobin studies indicate that the Hylobatidae diverged from other hominoids about 18 million years ago. About 7.5-4.5 million years ago, the diversification between African Hominoids occurred that would give rise to homo on one side and the African apes (gorilla and chimpanzee) on the other.

Man, chimpanzee and all Cercopithecoidea (monkey) tested so far have an ability to taste the sweet compounds: acesulfame-K, aspartame, D-tryptophane, sucrose, xylitol, monellin and thaumatin. In man and chimpanzee, but not in monkeys, gymnemic acid (GA) suppresses or abolishes the chorda tympani proper nerve (CT) response of these compounds. It is likely that at some time during evolution, the great apes and man acquired sweet receptors sensitive to the effect of GA which don't exist in monkey. The question asked in this study is: what is the effect of GA in the lesser apes? Recordings were made from the CT during stimulation with these compounds and NaCl, quinine and citric acid before and after GA. Besides amplitudes of the summated response other parameters were measured.

The results suggest some suppression, but not the same as in other Hominoidea by GA on the taste response. The results also show varying effects by GA on the responses to non-sweeteners. To summarize, in gibbon, GA seems to exert effects that fall between those of the greater apes and the Cercopithecoidea.

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Structure-Activity Relationships of Chemical Stimuli of Feeding Behavior by the Western Atlantic Ghost Crab Ocypode quadrata (Fabricius). THOMAS J. TROTT (Boston University Marine Program, Boston University)

The chemical response spectrum of the ghost crab Ocypode quadrata is sensitive to carbohydrates, amines, and organic acids. This investigation surveyed related compounds to determine the general structural features that confer activity for stimulating a stereotype feeding response i.e., cheliped flexion. The response of ghost crabs to carbohydrates was generally specific, disaccharides being more stimulatory than either mono- or trisaccharides. The disaccharides tested all contained a glucopyranose ring as one member of their composit sugars. The possession of an α-glucopyranoside linkage does not appear to be required for activity. Monosaccharides lacking C_6, the pentose sugars xylose and arabinose, were very potent stimuli, indicating that this carbon is not essential for activity. However, when C_6 is present, its acidification as in glucuronic acid, drastically lowered the effectiveness of the stimulus. The orientation of hydroxyl groups on carbons 1, 2, 4, and 5 was not critical. A free hydroxyl group on C_2 appears to be required; when amino groups were substituted in this position (glucosamine, mannosamine), the effectiveness of the stimulus significantly decreased. Linear molecules (sugar alchohols) were least effective stimuli, suggesting the importance of a hemiacetal ring. Amines < 4 or > 5 carbons were weak stimuli. Diamines of either 4 or 5 carbons were very potent when the amine groups were terminal only. Methylation of these amine groups significantly decreased the stimulus' effectiveness. Mono-, tri-, and tetra-amines were weak stimuli. Butanoic acid was most stimulatory, in contrast with other organic acids having either 3 or 5 carbons (proprionic and pentanoic acids, respectively). Amination of these acids had a differential effect depending on the position and number of amine groups. γ-Aminobutyric acid was slightly less potent than butanoic acid, but 3 times more stimulatory than α-aminobutyric acid. The diamines, 2,4-diaminobutyric acid and 2,3-diaminoproprionic acid were weak stimuli at best.

The Physico-chemical and Psychophysical Frontier of Sweetness

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Physico-chemical (solution) and psychophysical properties of various sweeteners have been studied to elucidate the structure/activity relationships in sweetness. The solution properties studied are apparent molar volume (ϕV) and surface tension (γ). These show fine distinctions between sweeteners which are in many cases attributable to their structural differences, but fail to account for the marked taste differences. The relationship between ϕV and γ for a particular sweetener may be an issue of stereostructure of some significance in taste chemoreception. The three dimensional relationships between concentration, ϕV and γ for selected sweeteners are therefore illustrated. The surfaces clearly give much more information about solute-water interaction than do the simple 2-dimensional graphs and emphasise the large differences in solution behaviour of different molecules over similar concentration ranges. When the 3-dimensional graphs of concentration and sensory properties (i.e. intensity - time) are constructed for the same sweeteners, similarities in the shapes of the surfaces are noted. For instance, structural analogues of hexoses and pentoses bear the same the 3-D relationship between concentration, intensity and time as noted for D-fructose - D-arabinose, D-glucose - D-xylose and D-galactose - L-arabinose. Moreover, Acesulfam-K has a similar 3-D plot of concentration, ϕV and γ as D-glucose and D-xylose and accordingly exhibits a similar surface for the 3-D plot of concentration, intensity and time. Thus, although solution properties may not be directly related to sensory properties, it seems more likely that intensity-time-concentration relationships are due to corresponding solution properties-concentration relationships. This approach may lead to a clearer understanding of the mechanism of taste chemoreception.

Electron microscopical demonstration of lectin binding at taste buds and surface mucus of the barbels of Silurus glanis (Teleostei). A preembedding and postembedding study.

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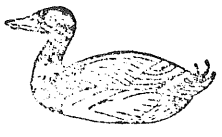
The mucus protecting the taste buds (TB) against mechanical and osmotic damage is also of some importance in recognizing and accepting special taste substances. To obtain more histochemical information about the carbohydrate composition of the TB mucus and the TB sensory cells themselves, we applied gold-labeled lectins to ultrathin sections cut from barbel-TBs of the European catfish, Silurus glanis (postembedding technique). Moreover, in order to see whether exogenous lectins can be transported via endocytosis into TB sensory cells or not, small pieces of barbels were incubated in different lectin-gold conjugates. Subsequently the specimens were fixed, embedded in epoxy resin and then cut (preembedding technique). For detection of particular sugars, in both procedures the lectins from Helix pomatia (HPA), Dolichos biflorus (DBA), Arachis hypogaea (PNA, all specific for galactosamine (GalNAc)) and Canavalia ensiformis (Con A, specific for glucosamine (GlcNAc) and mannose (Man)) have been used.

The postembedding studies done on thin sections revealed that only HPA binds to GalNAc residues, predominantly in vesicles of the TBs marginal cells and dark sensory cells. Also a few light sensory cells are marked by HPA which suggests a different state of development or function of these cells. Other lectins do not bind. The preembedding studies show that no lectin is penetrating into cellular compartments. HPA and Con A bind mainly to carbohydrate residues of the mucous layer on the top of the marginal cells and -rarely- to the apical plasmalemma of these cells. These results show that apparently particular carbohydrate components of the surface mucus may prevent the lectins from penetrating into the cells. Therefore, the TBs surface mucus may be understood as a structure which possibly serves as a sugar selective barrier, prior to the process of chemoreception.



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