ACHEMS-1990

THE TWELFTH ANNUAL MEETING OF THE ASSOCIATION FOR CHEMORECEPTION SCIENCES

BOOK OF ABSTRACTS

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ACHEMS - 1990

THE TWELFTH ANNUAL MEETING OF THE

ASSOCIATION FOR CHEMORECEPTION SCIENCES

ABSTRACTS

This book contains abstracts of the volunteer papers and posters of *ACHEMS-1990*. Abstracts are listed in order of presentation at the meeting. The abstracts for slide presentations precede the abstracts for poster presentations which were scheduled concurrently. An author index follows the abstracts.

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Age-Related Deficits in Odor Recognition Performance as a Function of Retention Interval: Sensory or Cognitive? MAGDALENA M. GILMORE and TRYGG ENGEN (Brown University).

The present paper addresses three questions. First, how much of the elderly's decreased ability to recognize common odors is a result of sensory deficits and how much is related to their cognitive capabilities? Second, can one facilitate odor recognition performance in the elderly by providing the semantic labels to the subjects while they simultaneously smell the odors? Third, will odor recognition performance decrease as a function of retention interval differentially for young and old adults? Participants, 35 elderly subjects (M = 71 years) and 35 young subjects (M = 21 years) performed a four-alternative odor recognition test. Subjects were randomly assigned to one of three conditions: 1) simultaneously smell and identify odors, 2) identify odors 15 seconds after smelling them, or 3) identify odors 30 seconds after smelling them. In addition, an olfactory threshold test and a cognitive status examination were administered to each subject. Overall, the elderly recognized fewer of the odors than did the young subjects. Olfactory thresholds and cognitive status were both significantly related to performance; removing the variance associated with each variable did not eliminate the significant age differences in odor recognition performance. In the simultaneous condition, when the cognitive requirements of the task were minimal, the elderly recognized 80% of the odors (the young subjects recognized 96% of the odors). The performance of the two age groups decreased differentially: the young subjects exhibited a gradual drop in performance from 96% correct to 81% correct across the 30-second retention interval, whereas the elderly showed a precipitous drop after 15 seconds to 56% correct. Cognitive factors, as indicated by the effects of retention interval and cognitive status, profoundly influenced odor recognition performance; therefore, minimizing the cognitive requirements of the task by allowing subjects to smell the odors while identifying them, will provide a more accurate assessment of olfactory functioning in the elderly.

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The Human Sense of Smell Has a Limited Capacity For Identifying Odors In Mixtures.

D G LAING CSIRO Food Research Laboratory, B A LIVERMORE Macquarie University, GW FRANCIS CSIRO Food Research Laboratory

Since most odors in our environment, whether at home, work or at play, are complex and often consist of dozens even hundreds of odorants, a question of considerable significance concerns the number of odorants humans are capable of perceiving in mixtures. The present study addresses this question using untrained and trained panels of laypersons, and an expert panel of perfumers and flavorists. A computer controlled air dilution olfactometer was used to deliver stimuli consisting of between 1 and 5 odorants, from a pool of 7 odorants, to subjects. The stimuli were common, dissimilar ordorants of equal moderate intensity and each was a single chemical. The results were unexpected and dramatic and showed that (1) regardless of training and experience, less than 5% of judgements of mixtures containing more than 3 odorants were correct and (2) regardless of whether judgements were correct or incorrect, subjects rarely selected more than 3 odorants, indicating that perceived complexity did not increase with mixtures containing more than 3 odorants. Overall, the results indicate that the human sense of smell is very limited in its capacity to discriminate and identify components of mixtures and this capacity is limited to 3-4 odorants. It is proposed that identification of the constituents of mixtures is limited by the convergence of multiple neural inputs at higher centres eg. olfactory cortex but be aided to some extent by temporal separation of input from each odorant

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Conditioned "Taste" Aversions in Humans: Are They Olfactory Aversions? LINDA M. BARTOSHUK (Yale University), JEREMY M. WOLFE (Massachusetts Institute of Technology).

Taste paired with nausea produces powerful conditioned aversions in rats. In humans, conditioned aversions have been formed experimentally in patients who experience nausea as a side-effect of chemotherapy and the natural occurrence of conditioned aversions has been studied by questionnaire. We present an analysis of the attribute(s) of the foods and/or beverages involved in 228 conditioned aversions described by students and staff at Yale and MIT. Participants were asked to describe the aversion. The smell was described as unpleasant for 29%, texture for 9%, the sight of the item for 13%, color for 4%, and even the thought of the item was said to be unpleasant in 13% of the aversions. Participants were also asked to provide a list of the foods and/or beverages to which their aversions had generalized. The generalizations suggest that olfaction plays a role in the aversion even when the smell is not explicitly mentioned (e.g., an aversion to orange-flavored baby aspirin generalized to all orange flavors). Most of the conditioned substances could be classified into three categories: 67% single food items (e.g., dill pickle), 29% two-component items (e.g., pancakes with maple syrup), and 11% complex items (e.g., lasagna). Of the two-component items, 81% generalized only to one component. Of the complex and single items, about half failed to generalize. Some of the generalizations were too complex to permit any simple sensory explanation. For example, one respondent reported an aversion to cheese crackers that generalized to vanilla wafers because the containers were similar. We conclude that conditioned food aversions are much more likely to be conditioned to smell than to taste in humans but that other attributes of the food condition as well.

Supported in part by NIH Grant DC00283

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Role of Olfaction in Perception of Nontraditional "Taste" Stimuli. THOMAS P. HETTINGER, WALTER E. MYERS and MARION E. FRANK (University of Connecticut Health Center, Farmington, CT)

The classical concept of human taste sensation as comprising only sweet, salty, sour and bitter has been challenged over the years by reports that certain substances have "taste" qualities not fitting neatly into these four categories. Qualities such as alkaline, soapy, sulfurous, metallic and umami have been entertained at one time or another to be genuine, and possibly unique, tastes. We hypothesized that, if olfaction played a role in these flavor perceptions, the use of nose clamps to reduce olfactory input would significantly alter the distribution of quality descriptors given for nontraditional "taste" stimuli. We asked 12 normal subjects, using a list of 9 possible descriptors (sweet, salty, sour, bitter, soapy, metallic, sulfurous, tasteless, other), to characterize the flavors of 1 mM L-cysteine, 1 mM sodium oleate, 1 mM ferrous sulfate and 100 mM monosodium L-glutamate (MSG), both with nose open and clamped. Water, 100 mM sodium chloride and 100 mM L-serine were used as controls. We found significant differences (chi-square, $p < .05$) in flavor profiles between nose open and clamped for L-cysteine, sodium oleate and ferrous sulfate, but not for MSG or the controls. A majority of subjects rated L-cysteine as sulfurous, sodium oleate as soapy and ferrous sulfate as metallic with nose open, but tasteless when clamped. The main descriptor for MSG was salty, with nose either open or clamped. We interpret these results as indicating that the characteristic flavors of L-cysteine, sodium oleate and ferrous sulfate are likely due to olfaction and that there is little basis for suggesting unique or fundamental gustatory qualities for these stimuli. An olfactory component for the umami flavor of MSG was not found, though it may have been obscured by strong gustatory components.

Supported by Clinical Center Grant DC00168 from NIH.

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Human Obesities and Sensory Preferences for Sugar/Fat Mixtures: Effects of Weight Cycling. ADAM DREWNOWSKI, CANDACE KURTH and JO RAHAIM (School of Public Health, University of Michigan, Ann Arbor, MI).

A representative group of 61 obese men and women (mean age 36 yrs; BMI=32.9) and 31 lean controls (mean age 34 yrs; BMI=21.5) recruited from the community-based Ann Arbor Diet Study tasted and rated 9 frosting-like mixtures of butter, sucrose, water and polydextrose. The samples contained 15, 25 or 35% fat and between 20 and 70% sucrose wt/wt. The subjects also tasted and rated a range of sucrose solutions in water. There were no significant group differences in taste perceptions or preferences for sweet solutions or sweet frostings between the obese and lean subjects. However, analyses by obesity subtypes revealed significant differences in sensory preferences for sugar/fat mixtures as a function of age of onset of obesity (childhood vs. late-onset) and dietary restraint scores. Childhood-onset obese dieters in the top restraint quartile showed the highest sensory preferences for sugar/fat mixtures. This group also showed greatest weight fluctuations during the year (mean 26 lbs.) and had recently lost a mean of 11 lbs. Analysis of 24-hr food recalls revealed further evidence of dieting and caloric restriction to lose weight among early-onset obese dieters. These data confirm anecdotal reports that obese individuals with a history of weight cycling ("yo-yo" dieting) show elevated preferences for sweet fat-rich desserts. The data are also consistent with reports of enhanced selection of a high-fat diet following overfeeding and deprivation cycles in laboratory rats. Supported by NIH Grants DK37011 and DK38073.

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Time-quality Tracking: Temporal Patterns of Taste Quality. S.A. ZWILLINGER[3], S.T. KELLING[1,2], B.P. HALPERN[1,2,3] (Physiology[1], Psychology[2], NBB[3], Cornell University, Ithaca NY 14853-7601 USA)

Changes in the presence or absence of taste qualities over time were studied using a 23 item single-letter computer keyboard code. Four screened female subjects with typing speeds of $\geq$ 45 words/min @ 95% accuracy learned the code until responses to computer monitor displays of the code words elicited 100% correct responses with reaction times $\leq$ 550 msec. In practice sessions subjects were instructed to track the taste of the liquid flowing over their tongue. Key presses were displayed on the computer monitor. Stimuli were 1 sec durations of distilled water, 2 mM Na-saccharin (NaSac), 10 mM citric acid in 2 mM NaSac (ArtLem), or 214 mM MSG, in random order, flowed over 39.3 mm^2 of the anterodorsal tongue tip region by a closed stimulus system. Practice continued until performance criteria were reached. Twelve data collection sessions followed. **RESULTS**: Duration of taste quality was much shorter than duration of previously reported tracked taste intensity (*Chem. Senses* **13**: 702, 1988; **14**: 716, 1989): median quality duration for NaSac, 594 msec; intensity duration, 1500 msec. Quality descriptions were similar to previous (*Chem. Senses* **13**: 559, 1988) vocal quality identifications: NaSac was 75% sweet, 6% sugar; ArtLem, 43% sour, 20% citrus, 11% sugar; MSG, 28% salty, 14% sour, 9% tomato. MSG gave two different qualities, separated by a no taste gap on 16% of trials. **CONCLUSIONS**: Temporal aspects of taste quality differ from intensity, supporting a concept of separate CNS processing for taste quality and intensity.

Supported by NSF Grant BNS-8518865 and UMAJ.

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Towards Quantitative Analysis of Gusto-and Nasofacial Reflexes. STEINER,J.E.(Dept.Oral Biol. The Hebrew Univ.-Hadassah Faculty of Dental Med.Jerusalem, Israel).

Gustatory and olfactory stimuli are adequate to unlock innate, reflectory, expressive orofacial motion-coordinations, the Gusto- and Nasofacial Reflexes. Since different stimulus-qualities activate different sets of muscles, facial expressions reliably indicate the organism's motivation towards the hedonic tone of the triggering stimulus (1,2). Further observations revealed,that motion features can be indicative not only of stimulus qualities but of intensities too,when reactions were videotaped and analyzed,using a notational-system,appropriate for quantification of individual motion components (3). In recent experiments these findings were reconfirmed and related to simultaneously occurring, differential heart-rate acceleration, induced by different tastes and their intensities. Interstimulus rinses had only little effect on the temporospatial organization of the facial response, but did facilitate the cardiac reactivity of infants to tastes. We demonstrated,furthermore, that tastes and odors trigger differential facial responses in those autistic children, whose verbal expressiveness is absent or reduced. Testing the elderly evinced that Alzheimer-type patients still display differential facial play indicative of pleasure-aspect of tastes and odors. Their catalog of features is limited as compared to that of normal age-mates or to that of infants. Finally, a similar analysis of facial expressive reactions to tastes and odors in members of primitive tribes - recorded by I.Eibl-Eibesfeldt - revealed that the facial play of these subjects is equally differential,as compared to those from western cultures, with response-profiles similar to caucasian perinatal infants reaction-patterns.

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2. Steiner,J.E. (1979) in:Adv.Child Devel.Vol.13. Eds.Reese & Lipsitt, Acad. Press.
3. Ganchrow,J.; Steiner,J.E.; Daher,M. (1983) Inf.Behav.Dev. 6:189-200

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Preferred Salt Concentration in a SouthWestern Sample of 200: Differences among Anglo and Hispanic Preschoolers and Their Parents. CLAIRE MURPHY, KRISTY STRAITS, RANI NIJJAR, JILL SNIFFEN, LISA TSUMURA, SAMUEL JINICH & MARICELA LEON-FUENTES (San Diego State University, San Diego, CA)

The purpose of the present study was to characterize salt preference among 50 Anglo and 50 Hispanic preschool children and their parents. All participants tasted three different diluents (deionized water, low-sodium chicken soup, and no-sodium V-8 juice) mixed with five different concentrations of salt (0.0,.05,.10,.20, and.40M NaCl). The participants were trained individually to use a bipolar line scale (visual analog scale) with a zero midpoint and no endpoints to rate their taste judgements of the pleasantness or unpleasantness of the stimuli. Training for this task for the preschoolers began with the children indicating by the span between their hands how much they liked or disliked self-reported familiar foods. The children then built lines on the visual analog scale with wooden toy blocks, to indicate taste preferences. Finally, they drew lines on the scale to correspond with preferences. Training was complete when the child demonstrated competence in drawing lines to indicate taste ratings for samples of each diluent that corresponded with ratings made by hand demonstrations and paired comparison ratings. The stimuli were then presented twice, in random order, and the line scale data were obtained. These data yielded both the peak preferred concentration (PPC) of salt for a diluent and the relative degree of preference for one stimulus over another. The data were subjected to ANOVA, with repeated measures. Results suggest that both the child's gender and the child's ethnicity play a role in salt preference. Preschoolers had higher salt preferences than adults. The results will be discussed in light of previous data from this laboratory characterizing salt preference in Anglo and Hispanic teenagers, and in Anglo, middle-aged and elderly adults. Because a preference for and ingestion of high concentrations of salt puts persons with hypertension at risk for coronary disease, a better understanding of the development of salt preference may suggest strategies for behavioral interventions which will ultimately reduce the incidence of coronary disease.

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GABA-like Immunoreactivity in the Gustatory Zone of the Nucleus of the Solitary Tract in the Hamster: Light and Electron Microscopic Studies. BARRY J. DAVIS (University of Alabama at Birmingham)

The purpose of the present study is to explore further the association between putative neurotransmitters and/or neuromodulators and the various classes of neurons within the rostral, gustatory portion of the nucleus of the solitary tract (NST). GABAergic somata and fibers have been identified in the caudal, non-gustatory NST and probably influence cardiovascular regulation. GABA's role in the gustatory NST is unknown but an association with a specific population of relatively small neurons could indicate the presence of inhibitory local circuit neurons. The distribution of GABA-like immunoreactive somata was studied in the gustatory zone of the NST in the hamster at the light and electron microscopic levels. At the light microscopic level, diffusely labeled immunoreactive somata that were mostly ovoid in shape and puncta were observed throughout the gustatory NST. Immunoreactive primary dendrites were occasionally seen. No immunoreactive fibers were observed. At the electron microscopic level, 18% of the neurons encountered were immunoreactive. All neurons possessed invaginated nuclear profiles and the average somal area was $85.5 \pm 2.8\ \mu m^2$ (12.7 x 8.4 μm; N=72), features that are characteristic of the most common class of neurons within the gustatory NST. GABA-like immunoreactive neurons appear to be smaller members of this class. The range of somal areas was narrower than that reported for tyrosine hydroxylase-like immunoreactive somata in the gustatory NST, suggesting a subset of GABAergic neurons that could possibly subserve a single function. Co-existence of a catechol (i.e., dopamine) and GABA in the same neuron can not be excluded at this time. Since GABA is often implicated as the neurotransmitter of small, inhibitory local circuit neurons, these findngs indicate a possible inhibitory aspect to the processing of taste information at the level of the first relay in the brainstem.

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Intracellular Recording from Brainstem Taste Neurons. MARTHA MCPHEETERS, THOMAS P. HETTINGER, LAWRENCE D. SAVOY and MARION E. FRANK (University of Connecticut Health Center, Farmington, CT)

Taste-responsive neurons of the hamster solitary nucleus have diverse chemical sensitivity, morphology and connectivity. In an attempt to directly relate structure and function at the brainstem level of processing in the gustatory system, we have made intracellular recordings from taste-responsive neurons of the solitary nucleus and characterized the cells histologically following injection of biocytin into the recorded cells. Biocytin is a new intracellular marker with notable advantages over HRP (horseradish peroxidase) [Horikawa and Armstrong (1988) J. Neurosci. Methods 25, 1-11]. We recorded from 7 medullary taste neurons having membrane potentials of at least -40 mv using microelectrodes containing 2% biocytin in 2 M potassium acetate with impedance values of 50-250 megohms. The neurons could be stimulated to produce action potentials by application of taste solutions to the tongue and by brief depolarizing current pulses. Impalements lasted up to 20 minutes. Three neurons were successfully filled with biocytin and reconstructed from serial sections following visualization of the biocytin with an ABC (avidin-biotin complex) reaction. They were characterized as either stellate or elongate neurons and had somal areas between 150 and 300 um^2. One particularly elaborate stellate neuron was seen to have a dendritic field that extended throughout the rostral pole of the solitary nucleus and had an axon that could be followed to terminals in the parabrachial nucleus in the pons. Functional and morphological characteristics of these neurons were consistent with previously obtained extracellular recordings and neuroanatomical studies of the solitary nucleus. Recordings from identified and fully reconstructed neurons are necessary to develop precise hypotheses about the role of cellular organization in signal coding in the solitary nucleus.

Supported by the University of Connecticut Health Center.

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Separation of Neuron Types in the Gustatory Zone of the Rat Solitary Nucleus Based on Intracellular Electrophysiological Characteristics. ROBERT M. BRADLEY AND ROBERT D. SWEAZEY (Dept. Biologic and Materials Sciences, School of Dentistry, University of Michigan).

Investigators using morphological techniques have shown that several neuron types exist in the rostral nucleus of the solitary tract (NTS). We now report that neuron types in the NTS can be distinguished using biophysical techniques. Intracellular recordings were made from 51 NTS neurons using glass micropipettes in coronal brainstem slices prepared with standard techniques. For each neuron intrinsic membrane properties were measured and current-voltage plots constructed. Neuron types were separated using two current injection paradigms. In the first, neurons were depolarized from resting membrane potential; in the second, an initial hyperpolarizing pulse of varying length and magnitude was followed by a long depolarizing pulse. Based on responses to these paradigms three neuron types could be distinguished. Type I neurons had a low frequency, regular pattern of response to depolarizations, and the first spike initiated by depolarization could be delayed by a hyperpolarizing prepulse. This delay was linearly dependent on the magnitude or duration of the prepulse. On the other hand, Type II and III neurons had a high frequency response to depolarizations, and the first spike initiated by depolarization could be delayed by a hyperpolarizing prepulse. However, the delay was a non-linear function of prepulse magnitude or duration. Furthermore, Type II neurons had a regular pattern of response to long depolarizations whereas Type III neurons had a burst pattern. The different membrane properties of these neuron types are important in determining responses to afferent input and may indicate that these neurons have very different roles in the gustatory pathway.

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Taste, tactile and gastric inputs converge onto multimodal neurons in the medulla: Analysis of single units from multiunit, extracellular recordings. JAGMEET S. KANWAL (Dept. of Cell. & Struct. Biology, Univ. Colorado Sch. Med., Denver, CO 80262).

Behavioral and physiological data suggest that gustatory and gastrointestinal inputs interact at one or more sites within the brain. However, neuroanatomical studies show that coelomic (general visceral) and gustatory inputs ascend in parallel but separately through the CNS. While "downstream" reflex-type pathways exist for the gustatory modulation of vagal motor output, a locus in catfish where gastric and gustatory inputs converge is unknown. Electrophysiological recordings were obtained from ventral medullary regions in the vicinity of the facial motor nucleus in the channel catfish, *Ictalurus punctatus*. HRP-filled, glass micro-electrodes (impedence < 1 megohm) were utilized to record in catfish in which stimulating electrodes had been implanted in the stomach. Once the HRP-filled, glass microelectrode encountered neurons responsive to taste and/or tactile stimulation, the stomach was stimulated electrically with previously implanted hook electrodes. If the neurons responded both to gustatory and to electrical stimulation, the recording site was marked with an iontophoretic injection of HRP. The extracellular multiunit activity was analyzed by separating the heterogenous population of waveforms into unique color-coded clusters of similar waveforms. Peristimulus-time-histograms were then generated for each class of waveforms. This indicated the presence of at least two types of multimodal neurons which responded both to chemical or tactile stimulation of the oral cavity and to electrical stimulation of the stomach. One neuron type exhibited a spontaneously rhythmic pattern of activity (average firing rate approx. 10 spikes/s), while the other had a relatively low rate of spontaneous activity (approx. 1 spike/s). Subsequent anatomical analysis confirmed that the gastrogustatory convergence site is located in the dorsolateral reticular formation at the level of the facial motor nucleus. Several small (approx. 30 μm and a few large (approx. 70 μm) neurons were labeled at the recording site.

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The Effect of Amiloride on Single Taste Neurons in Rat Nucleus Tractus Solitarius.
BARBARA K. GIZA, THOMAS R. SCOTT, ROBERT F. ANTONUCCI, and KATHERINE T. SPENCE (University of Delaware).

Amiloride is a potent inhibitor of sodium transport in a wide variety of systems. Applied to the tongue, it decreases both short-circuit current across the lingual epithelium and evoked neural activity to Na-Li salts by about 50%. We recorded the responses of 37 single neurons in the rat NTS to an array of taste stimuli before and after the application of $5x10^{-4}$M amiloride to determine the degree and specificity of inhibition. Across all cells, responsiveness to 0.1M NaCl declined by 47% following amiloride treatment. The effect, however, was starkly different for separate neural subtypes. Taste cells whose profiles indicated primary sensitivity to Na-salts alone or to Na-salts and sugars were profoundly suppressed by amiloride; those whose profiles showed broad responsiveness to NaCl, acids and bitter salts were unaffected. Thus taste cells in the CNS may be functionally separated and selectively identified with specific receptor mechanisms employed at the lingual epithelium. The effect of amiloride on the taste quality of Na-Li salts was to shift it sharply toward sour and bitter stimuli and away from those that are sweet.

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Olfactory input to the orbital cortex in the monkey, *Macaca fascicularis*.
J.L. Price, S.T. Carmichael and M.-C. Clugnet (Washington Univ. School of Medicine, St. Louis, MO 63110)

A combination of electrophysiological recording and axonal tracing methods have been used to define direct inputs from the primary olfactory cortex to the posterior orbital cortex, and possible indirect projections to more widespread cortical regions. Electrical stimulation of the olfactory bulb in two monkeys (under halothane anesthesia) evoked short latency unit responses in primary olfactory cortical areas (the anterior olfactory nucleus and the piriform cortex), and also in the ventromedial part of the agranular insular cortex (AIv) and the posteromedial part of orbital area 13 (area 13a), immediately lateral and rostral to the anterior olfactory nucleus, respectively. However, electrode penetrations rostral or lateral to AIv and area 13a did not reveal any response to olfactory bulb stimulation.

Retrograde tracer injections into AIv and area 13a labeled many cells in the piriform cortex. Other experiments with injections in more rostral or lateral parts of the orbital cortex labeled very few if any cells in the piriform cortex. In confirmation of this, an anterograde tracer experiment with an injection of ^{3}H-leucine in the piriform cortex labeled a substantial axonal projection to AIv and area 13a, but only very slight projections to more rostral or lateral areas. Another experiment in which the ^{3}H-leucine injection involved the piriform cortex together with the endopiriform nucleus deep to it produced a similar but slighlty more extensive pattern of axonal label.

These results indicate that direct olfactory cortical input to the orbital cortex is limited to a small part of the agranular insula and the posterior orbital cortex, immediately adjacent to the primary olfactory areas. Further axonal tracing experiments have shown that this region in turn projects to more rostral orbital areas, which may thereby receive less direct olfactory information. Because experiments in other labs have indicated that these more rostral areas respond to gustatory and visual stimuli it is likely that they are multimodal areas.

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Effect of Amygdala and Orbitofrontal Cortex Lesions on Taste Preferences in the Primate.
LESLIE L. BAYLIS (Dept. of Psychobiology, U. C. Irvine), and DAVID GAFFAN (Dept. of Experimental Psychology, Oxford University).

Previous studies have shown that the amygdala and orbitofrontal cortex perform a role in ingestive behaviors (eg. Aggleton and Passingham, 1981, *JCPP 95*, 961; Butter and McDonald, 1969, *Science 164*,1306). What is unclear is whether lesions affect the perception of stimuli, the affective response to stimuli, or motivation.

The two lesioned and one unoperated control groups were tested on their preferences of four foods in a task in which choices were made between two foods on a food-board. All four stimuli were novel to these monkeys, but designed to span from highly palatable (apple) through lemon and olive to highly aversive (raw meat). Both lesioned groups chose and ate more of the less preferred stimuli (olive, raw meat) than controls. However across all groups the preference *order* was the same. This suggests that lesioned monkeys had an intact sense of taste while showing less aversion to the non-preferred foods.

In a visual discrimination task in which correct responses were reinforced by juice, and errors by aversive saline, the performance of lesioned monkeys was close to chance. However, in a second discrimination task where only correct responses were reinforced, the performace of lesioned monkeys was similar to controls. This suggests that the lesioned monkeys were not differentially motivated by the positive and negative reinforcers in the first task, perhaps because both were perceived to have similar hedonic value.

Taken together these results suggest that lesions to the amygdala and orbitofrontal cortex, lead to a flattening of the hedonic contrast between taste stimuli.

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Taste-evoked Activity in the Insular-opercular Cortex of the Alert Cynomolgus Monkey.
THOMAS R. SCOTT, CARLOS R. PLATA-SALAMAN and VIRGINIA L. SMITH (University of Delaware).

The anesthetized rodent preparation has long provided a convenient neural model for taste. Gustatory systems in rodents, however, differ from those of primates with regard to neuroanatomy, in their relative sensitivities to the prototypical taste stimuli and in physiological responses to nutritional loads. Thus we sought to establish a preparation in which phylogenetic differences and the effects of anesthesia were minimized and so to create a more appropriate neural model for human gustation. In this abstract we provide a functional characterization of gustatory cortex in the alert cynomolgus monkey, and in the next seven, describe the initial data sets derived from it. Neurons responsive to taste stimuli were located over an area that included 95 mm^3 of insular-opercular cortex, within which they constituted 10% of the population. Intermingled with them were non-gustatory cells sensitive to 1) light touch within the oral cavity (5%), 2) tongue extension (2%), 3) other mouth movements (12%) and 4) stimulus approach (2%). The sensitivities of 70% of the neurons could not be determined. Taste-responsive cells generated small action potentials (<400uV) and had low spontaneous rates. Evoked activity was predominantly excitatory and tuning was rather broad ($\bar{H}$=0.71; N=503). There was no clear chemotopic organization in gustatory cortex. Recordings were quite stable, with a mean test-retest reliability coefficient over 30-60 min of +0.92. This preparation provides us with an alert, intelligent, dexterous subject whose sensory profile resembles that of the human (visually dominant, microsmotic) and with whom we can interact on a daily basis for 6-12 months. Thus neural and behavioral experiments may be conducted simultaneously, the number of subjects is minimized and the rate of data collection is high.

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The Formation of Bimodal Taste and Visual or Taste and Olfactory Neurons in the Orbitofrontal Cortex of Primates. Edmund T. ROLLS, Simon J.THORPE, Roger MASON, Edward A. WAKEMAN and Teresa L.WHITE (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 the orbitofrontal cortex some neurons are multimodal, and receive visual and/or olfactory inputs as well as gustatory inputs (Rolls and Wiggins, 1989 Chem. Senses 14:; Rolls, 1989 J. exp. Biol. 146: 141-164). 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, Rolls and Maddison, 1983 Exp. Brain Res. 49: 93-115), can form these multimodal neurons by learning. We report that for at least some of the bimodal neurons in the orbitofrontal cortex, the bimodal responses can be modified by learning. For example, some neurons in this region respond to visual stimuli only while the visual stimuli are associated with the delivery of the taste of sucrose in a visual discrimination task. Moreover, these neurons can reverse their responses in a reversal of the visual discrimination task, ceasing to respond to that visual stimulus when it becomes associated with saline, in as little as one trial. We conclude that at least some of the bimodal neuronal responses found in the primate orbitofrontal cortex can be formed and modified by learning.

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Behavioral Responses to Chemical Food Signals by a Salt Marsh Protozoan. M. LEVANDOWSKY (Haskins Labs, Pace University, N.Y., NY 10038). *

Videomicroscopy was used to quantify behavioral responses to chemical signals by the marine heterotrich ciliate, Fabrea salina. In the vicinity of agar plugs containing supernatant from cultures of the food algae Dunaliella salina, Isochrysis galbana and Rhodomonas lens, starved ciliates swam in atypical tight circles, leading to an accumulation. The inhibitaory algal species, Pavlova gyrans, did not elicit this behavior. The accumulation factor appeared to be dialyzable and heat-labile. Effects on the behavior were detected when cells were exposed to the heavy metals Pb, Ag at nominal concentrations that were 2 orders of magnitude below the levels where effects on survival and growth detected.

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Comparative Plant Recognition Systems of Eastern North American Limenitis (Lepidoptera: Nymphalidae). DAVID FLAIM (University of Maryland, Baltimore Co.)

Extensive literature surveys on the plant relationships of eastern North American Limenitis (Lepidoptera: Nymphalidae) species (L. archippus and L. arthemis) reveal specific differences in plant utilization. Larval foodplant ranges are more broad than the host plant ranges selected by ovipositing females. Investigation of the breadth of the larval foodplant and adult oviposition ranges resolve the discrepencies that are reported in the literature. The extent of the ranges may provide impetus for re-evaluation of the mimetic complexes of L. archippus with (Danaus plexippus) and L. arthemis astyanax (with Battus philenor). Behavioral plasticity, resulting from previous exposure to plants, allows L. arth. astyanax and L. arch. archippus larvae to display foodplant induction of preferences. Additionally, taxonomic closeness of plants is inversly related to the strength of the induction of preferences by these larvae. Certain extracts (ethanol for larvae and water for adults) from Salix babylonica foliage can stimulate feeding and oviposition behaviors in L. arch. archippus and L. arth. astyanax. Aqueous extracts of Prunus serotina foliage stimulate feeding in L. arth. astyanax larvae. Ablation assays implicate the importance of the chemosensory maxillary styloloconica for host/non-host plant recognition. At present, electrophysiological recordings from these chemosensory maxillary styloconica are underway and will provide information on the neurophysiological bases of the larval feeding behaviors. Examination of the hybrid offspring resulting from mating the oligophagous (L. arch. archippus) and the polyphagous (L. arth. astyanax) may lead to a description of a model for genetic inheritance of chemoreceptor sensitivities underlying the larval feeding behaviors.

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Endocrine Control of Feeding Behavior in Sand Fiddler Crabs. M. SEARS (Duke University Marine Laboratory).

Uca pugilator is a deposit feeding semiterrestrial crab. Feeding has been shown by others to be mediated via dactyl chemoreceptors. Hexose sugars stimulate feeding behavior. Eyestalk ablation (a means of removing neural as well as endocrine integrative centers) increases sensitivity of crabs to hexose stimulants.

The potential for endocrine control of feeding responses to chemostimulants was examined. Injection of eyestalk extracts and more specifically the sinus gland region of the eyestalk restores spontaneous feeding activity and sensitivity to hexoses to near normal. The time course of the change in responsiveness is similar to that observed for changes in chromatophores upon sinus gland injection. Chromatophores are known to be under endocrine control. These results suggest that the sinus gland secretes a hormone that inhibits feeding.

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Chemo-orientation of the Lobster, *Homarus americanus*, to a Point Source in a Laboratory Flume. NAT SCHOLZ, PAUL A. MOORE, LYNNE LACOMIS, and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

Many animals use chemical cues contained within turbulent odor plumes for orientation, but the relevant parameters of the plume are not known. Orientation studies in aquatic environments have advantages over those in terrestrial environments because odor distribution patterns can be more accurately measured and stimulus delivery more closely controlled. We chose the lobster, *Homarus americanus*, because of its size and ease of handling, and the extensive base of neurophysiological data on the properties of its chemoreceptor cells. Previous behavioral studies indicate that the lobster relies heavily on chemosensory input in its natural habitat and specifically on antennular chemoreception for efficient orientation (Devine and Atema 1982, Biol. Bull. 163: 144-153). In a flow-through flume (90 x 250 x 20 cm) lobsters oriented towards a stimulus (0.5g/l homogenized and centrifuged mussel tissue in raw seawater) constantly flowing from a pipette two meters upcurrent. Flow parameters were identical to those used for detailed plume description in Moore et. al. (Biol. Bull.) and Atema et. al. (AChemS abstracts 1990). Lobsters were placed on a mussel diet and then starved for at least three days prior to testing. Tests were videotaped with a camera mounted directly overhead on a moveable track; tapes were digitized at 1 Hz with the rostrum as the reference point. Orientation paths were analyzed for walking speed, absolute and relative magnitudes of turn angles, and absolute and relative magnitudes of headings. Preliminary results from this analysis favor the hypothesis of direct chemosensory control of orientation (i.e. "chemotaxis") rather than an innate behavior/motor program (such as zig-zagging) triggered by chemical stimulation.

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High-Speed Electrochemical Recordings With Microvoltammetric Electrodes: A New Tool For High-Resolution Analysis of Aquatic Odor Signals. GREG A. GERHARDT (Depts. of Psychiatry and Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262), PAUL MOORE and JELLE ATEMA (B.U.M.P., M.B.L., Woods Hole, MA 02543, USA)

Aquatic odor signals serve as sources of information for many marine animals during orientation and foraging. However, the detailed structure of aquatic odor signals has remained unclear due to inadequacies in chemical sampling technologies. Clearly, there is a lack of information regarding high-resolution spatial and temporal properties of odor signals relevant to chemoreceptor cells and organ function. Using high-speed electrochemical techniques coupled with microvoltammetric electrodes, we have recently been able to investigate odor signals of tracer molecules with spatial resolutions of 10 to 150 microns and temporal properties of as rapid as 200 hertz (Moore et al. 1989, Chem. Senses 13: in press). The electrode recording concepts are based on controlling chemical reactions of a tracer molecule, such as dopamine, at the surface of carbon-based electrodes. The generated current flow due to the oxidation of molecules that strike the surface of the probe is linearly related to the number of molecules that have come in contact with the electrode. This recording technique has been applied to studies of aquatic odor signals in recirculating flumes, recording chambers and deep ocean conditions. Our preliminary studies support that these new recording techniques can be used to characterize high-resolution properties of odor signals with the necessary spatial and temporal resolution needed for the more detailed understanding of chemoreceptor structures.

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Taste-dependent selection and rejection by toads. STEVEN T. KELLING, BRUCE P. HALPERN (Cornell Univ. Physiology/Psychology/NBB. Ithaca NY 14853-7601)

Although the anatomical and physiological properties of the anuran gustatory apparatus have been frequently studied, it is unknown whether anurans make behavioral decisions based on their sense of taste. A bioassay was developed to determine if toads could make taste related decisions. METHODS Toads (Bufo americanus n=12) were individually placed in a (10 x 14 x 10 cm) rectangular enclosure. Agar pellets (3 mm x 7 mm) were slowly moved in front of the toads using a mechanical transport system. Three types of agar pellets were used: distilled water control pellets, mealworm exoskeletons in distilled water (MW), and the superficial skin layer of B. americanus in distilled water (TS). Each session consisted of 3 trials: DW, a stimulus (MW, TS, or DW), DW. Each session containing a MW or TS trial was followed by a session where all trials were DW. Each toad participated in 3 sessions with MW and DW, and 6 sessions of DW. There was a 48 hour intersession interval. After 24 hr, toads were fed mealworms ad libitum. Measurements from high resolution video tape were made of the number of strikes (retrieval of pellet into buccal cavity), swallows, and rejections (spitting out of a pellet from the buccal cavity). MW was placed in the enclosure during all sessions to control for any olfactory cues. RESULTS Toads would strike DW pellets with the same frequency (80%) as either MW or TS pellets. However, toads swallowed DW pellets during 50% of the strikes, MW pellets 74%, and TS 22% of the strikes. The number of strikes during TS and MW were significantly different (chi-square $p>0.001$) from DW strikes. CONCLUSIONS Toads appear to make behavioral decisions based on the sense of taste. This will be confirmed with peripheral nerve lesions.

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Microstructure of Salt Intake During Successive Sodium Depletions S.P FRANKMANN, C.J. O'CONNOR, G.P. SMITH (NYH-Cornell Med. Ctr.) and J.D DAVIS (Univ. of Illinois, Chicago)

After recovery from a first sodium depletion, salt intake to a second depletion increases approximately two fold (1). To determine the changes in the pattern of licking that underlie the increased intake, male, Sprague-Dawley rats were sodium depleted (Lasix, 10 mg) and 24 h later their intakes of 0.3 M NaCl were recorded for 1 hour using a computerized, lickometer, data-collection system. Each time the tongue of the rat contacted the sipper tube, a circuit (60 nanoamp) was closed and the lick was recorded (10 msec resolution). At the end of the session, the licks were analyzed for rate, size of burst (SB), and length of the interburst interval (IBI). Minimum IBI was > 0.25 sec and the minimum burst size was 2 licks. These data were collected and analyzed for three successive sodium depletions (each separated by 1 week).

Dep #	Intake (ml)	Licks/min (1st 4 min)	SB (sec)	IBI (sec)	Bursts (no.)	Duration (min)
1	11.3	155.8	16.2	5.8	138.7	16.5
2	9.6	216.2*	14.9	3.9	123.7	12.7
3	17.3*	226.8*	20.9	5.1	175.0*	26.2*

(All values represent the mean (n=3). * = $p < .05$ vs 1st depletion.)
Intakes of the 0.3 M NaCl were increased at the third but not the second sodium depletion. Analysis of the rate of licking revealed increases at both the second and third depletions. Thus, the increased rate of licking is not sufficient to increase total intake. At the third sodium depletion, the number of bursts and the duration of the intake were increased in addition to the rate of licking. Thus, the increased intake of 0.3 M NaCl at the third depletion was accomplished by increasing the duration of intake by increasing the number of bursts of drinking. In comparison with previous data collected with varying concentrations of sucrose (2), these data are consistent with the saline solution being more palatable at successive sodium depletions.

1) Behav. Neurosci. 101: 724, 1987 2) Appetite 11: 229, 1988
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Changes in Blood Pressure and Urinary Electrolyte Levels With Dietary Obesity and Weight Cycling in Rats. ROBERT J. CONTRERAS, SANDI KING, LAWRENCE RIVES, AMY WILLIAMS, & TIFFANY WATTLETON (University of Alabama, Department of Psychology, Birmingham, AL)*

In humans, a diet of rich and palatable foods and weight cycling ("yo-yo" dieting) are feeding patterns frequently associated with obesity and possibly, hypertension. Inasmuch as little is known about mechanisms, we have tried to develop an appropriate animal model of obesity-associated hypertension. The aims of the present study were to: (1) replicate the findings of Ernsberger and Nelson (*Am. J. Physiol.* 254: R47-R55, 1988) reporting the development of mild hypertension in dietary obese Sprague-Dawley rats exposed to 4 cycles of food restriction-refeeding (weight cycling) on a sweet milk diet; (2) determine whether this mild hypertension was associated with changes in sodium regulation, hyperinsulinemia, and in angiotensin and autonomic control of blood pressure. Twenty-five male Sprague-Dawley rats were divided among three groups. Eight rats were fed Agway pelleted chow (Pellet Group), and 17 rats were fed pelleted chow plus sweetened condensed milk diluted with water and fortified with vitamins and minerals. After 4-wk, the milk-fed rats were divided into two groups matched in weight and blood pressure. Eight rats were continued on the same sweet milk diet (SM Group), and 9 rats were exposed to four cycles of a 4-d fast alternated with 2-wk of refeeding the pelleted chow and sweet milk diet (Cycled Group). Blood pressure and heart rate (HR) were measured by the tail-cuff technique at the onset of each fast, on the last day of the fast, and after 3 days of refeeding. During fasting 24-h measurements of urine volume and urinary sodium and potassium concentration were also obtained. Four weeks after the last fast, baseline mean arterial pressure (MAP) and HR, and MAP and HR responses to 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. One day later, plasma samples were taken from 4-h fasted rats for assessments of plasma sodium, potassium, glucose, and insulin. The animals were then sacrificed and their heart, kidneys, adrenals, retroperitoneal white fat pad, interscapular brown fat pads were removed and weighed. These data are currently being analyzed.

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Preference for Fat-Rich Foods is Related to the Level of Fat in the Rearing Diet. WARWICK, ZS and SCHIFFMAN, SS (Duke University)

Preferred levels of NaCl, sucrose, protein and non-nutritive flavors by adult humans and rats are related to the dietary history of the individual. The purpose of the present study was to determine whether the quantity of fat in the rearing diet was related to subsequent patterns of ingestion. Beginning at 21 days of age female rats were fed either a High Fat (HF) diet (30% of calories from fat) or a Low Fat (LF) diet (10% of calories from fat). Safflower oil was used as the fat source and was manipulated at the expense of carbohydrate. Short-term preference tests were conducted after 4 weeks on the diet. Test stimuli were 3 peanut-butter based mixtures that differed in fat content. Intake of NaCl and sucrose solutions was also measured. Rats raised on a HF diet had greater total consumption of test foods relative to consumption by animals raised on a LF diet. The difference in total intake was mainly attributable to greater ingestion of the highest-fat mixture by the HF group. During a separate test session, LF group animals consumed more of NaCl solutions (0.9% and 1.8%) than HF group animals. HF and LF groups did not differ in their ingestion of sucrose solutions nor in body weight gain. Half of the animals in each group were then fed the alternate diet, while the other animals remained on the accustomed diet. This yielded four groups (early diet/subsequent diet): LF/LF, LF/HF, HF/HF, HF/LF. Preferred level of fat was assessed after 4 weeks. Groups HF/LF and HF/HF had greater total consumption of the fat-rich test foods than groups LF/HF and LF/LF. These findings suggest that ingestion of fat-rich foods during early development may induce shifts in taste preferences that are not readily reversible by subsequent consumption of a lower-fat diet.

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The Role of Sucrose-sensitive Neurons in Ingestion of Sweet Stimuli by Hamsters. BRADLEY G. REHNBERG, MARION E. FRANK & THOMAS P. HETTINGER. (University of Connecticut Health Center, Farmington, CT)

Substances preferentially ingested by hamsters (Mesocricetus auratus) can be considered sweet because they generalize to sucrose in conditioned taste aversion tests. We addressed the role of chorda tympani neurons in preferences for aqueous solutions of a variety of "sweet" stimuli. First we looked at how hamsters respond to the gustatory, olfactory, and irritant oral stimuli: 0.1 M NaCl, 0.001 M quinine.HCl, 0.01 M citric acid, 0.001 M dithiothreitol, 0.01 M Na 2-mercaptoethanesulfonate (NaMES), 0.01 M pyridine, 0.01 M 2-phenylethanol, 0.005 M i-amyl acetate, 0.01 M vanillin, half-saturated l-menthol and 0.033 mM capsaicin. These stimuli were preferred in 2-bottle tests only if they were sweet or made sweet by adding 0.5 M sucrose. Since sucrose predominantly affects one of three physiologically defined populations of neurons in the hamster chorda tympani, activity in these "S units" may be a sufficient sensory signal for preference. Second, to determine if S-unit activity is necessary for preference, we presented to the tongue a variety of stimuli while recording responses of single chorda tympani neurons. We used eight equally preferred, sweet stimuli: 0.03 M sucrose, 0.001 M Na saccharin, 0.01 M D-phenylalanine, 0.1 M glycine, 0.01 M Ca cyclamate, 0.005 M p-phenetylurea (dulcin), 0.003 M Na 3-nitrobenzenesulfonate (NaNBS) and 0.03 M NaMES; as well as two non-sweet stimuli: 0.03 M NaCl and 0.03 M NH_4Cl. Sucrose, saccharin, D-phe, gly, dulcin, and NaMES clearly activated S units whereas NaNBS and Ca cyclamate did not. Like NaCl, sweet sodium salts activated N units and, to a lesser degree, H units. Because NaNBS was tested at a low concentration (0.003 M), it was a weak stimulus for all chorda tympani fibers. Ca cyclamate was also a weak stimulus but it activated H units more than N units, like NH_4Cl. Thus, S-unit activation may be sufficient but not necessary for preference of sweet solutions. This suggests that primary afferents in other taste nerves and S units in the chorda tympani converge in the central nervous system.

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Independent Processing of Ingestive and Aversive Affective Taste Characteristics: A Detailed Analysis of Oral Motor and Somatic Taste Responses. PAUL A. S. BRESLIN, ALAN C. SPECTOR, HARVEY J. GRILL (University of Pennsylvania)

When the taste of sucrose is immediately followed by a LiCl injection, a rat's oral motor and somatic responses to the sucrose change when measured several days later. These responses are thought to reflect the affective characteristics of taste stimuli and have been divided into two domains: ingestive and aversive. Ingestive taste reactivity (TR) is composed of tongue protrusions (TP), mouth movements (MM), and lip flares (LF). Aversive TR is composed of gapes (G), chin rubs (CR), and forelimb flails (FF). This study evaluates the relative contribution of each of these components to the changes observed in sucrose elicited TR following sucrose-LiCl pairings. Sucrose elicited TR was recorded in 15 Sprague-Dawley rats both before and after either one, two or three pairings of sucrose with a LiCl injection. The animals were randomly assigned to one of three groups. Group 1 animals received one taste-LiCl pairing, group 2 received 2 pairings and group 3 received 3 pairings. A sucrose-LiCl pairing consisted of a 30 sec intra-oral infusion of 0.1M sucrose at 1ml/min immediately followed by a LiCl injection (i.p.) (3.0 mEq/kg). All pairings were separated by four days. All groups received a final sucrose test four days after their last taste-LiCl pairing. Their TR responses were videotaped for later analysis. Ingestive taste reactivity scores (TP + MM + LF) elicited by 0.1M sucrose significantly decreased and aversive taste reactivity scores (G + CR + FF) significantly increased as a function of the number of taste-LiCl pairings. Components within the ingestive domain showed differential sensitivity to the number of taste-LiCl pairings with tongue protrusions decreasing the most after one pairing, and lip flares and mouth movements decreasing the most after two pairings. Note that all three components decreased as a function of trials. Aversive components also showed differential sensitivity but the opposite trend. Gapes and chin rubs both increased significantly after the first pairing and again after the second pairing. Forelimb flails only significantly increased after the third pairing. All three aversive components increased as a result of taste-LiCl pairings. The ingestive TR score on the final sucrose test was significantly correlated with baseline ingestive behavior ($r = .64$). Aversive TR score was never correlated with ingestive score. However, aversive TR was not a random process, since the aversive score in trial one was significantly correlated with the aversive scores on subsequent trials ($r = .69$). In conclusion, it appears that all components within a TR domain show similar trends of increasing or decreasing, with some components demonstrating greater sensitivity than others to the number of taste-LiCl pairings. Furthermore, these findings suggest that the neural substrates underlying control of ingestive and aversive TR function independently.

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Ibotenic Acid Lesions of the Dorsomedial Hypothalamic Nuclei Induce Enhanced Taste Aversion Learning. SHIGERU FURUTA and DANIEL A. DEEMS (Smell & Taste Center, Department of Otorhinolaryngology and Human Communication, School of Medicine, University of Pennsylvania).

The dosomedial hypothalamic nucleus (DMN) has been implicated in the regulation of food intake, water intake and body weight regulation. Recently we have demonstrated that destruction of the dorsomedial hypothalamic region enhances conditioned taste aversions (CTA) by increasing sensitivity to illness. Since electrolytic lesions destroy both cell bodies and neuronal fibers, the present study evaluated the phenomenon using the cellular neurotoxin ibotenic acid, which destroys cell bodies, leaving neural fibers of passage intact. A lithium chloride-based generalized aversion to sodium chloride was used as the measure of illness induction. Twenty male Long Evans hooded rats served as subjects; 12 animals received ibotenic acid lesions of DMN (10 ug/ul, 150 nl), eight animals served as sham controls. Animals were habituated to a restricted drinking schedule in the home cage (20 min access to distilled water at 09:00 followed one hour later by 60 min free access to water). After 10 habituation trials animals received six aversion conditioning trials consisting of 20 min access to 15 mM lithium chloride brought to 100 mM with 85 mM sodium chloride. Generalized aversions to 100 mM sodium chloride were evaluated across six extinction trials. Results revealed that DMN lesioned animals demonstrated enhanced acquisition and delayed extinction of taste aversions compared to control animals. These results provide further evidence that DMN is involved in the mechanisms of responsivity to illness in the CTA paradigm.

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RNAs for Proline-Rich Proteins in von Ebner's Gland of Mice and Macaques and for Statherin and Histatins in von Ebner's Gland Containing Tissues of Macaques. E.A. AZEN, L.M. SABATINI (Dept. of Medical Genetics, Univ. of Wisconsin), G. HELLEKANT (Dept. of Veterinary Sciences, Univ. of Wisconsin) and T.F. WARNER (Dept. of Pathology, Univ. of Wisconsin, Madison).

Our main purpose was to search for expression of proline-rich proteins (PRPs) as well as the other salivary-type proteins, including statherin and histatins in taste bud tissues of mice and macaques. We began these studies because of previous genetic findings in mice that Prp and taste genes for certain bitter substances are either the same or closely linked (Azen, Lush and Taylor; Trends Genet. 2:199, 1986). Recent unpublished genetic data (in recombinant inbred and congenic strains of mice) obtained in collaboration with G. Whitney (Psychology Dept., Florida State Univ., Tallahassee) support this conclusion. We tested taste bud tissues for specific mRNAs with specific labeled DNA probes by northern blotting and in situ hybridization. We found expression of PRP mRNAs in von Ebner's gland of mice and macaques. There was a much greater degree of PRP mRNA induction in mouse parotid (16x) than in von Ebner's gland (2x) after in vivo isoproterenol stimulation. We also found statherin and histatin mRNAs in macaque taste bud tissues containing von Ebner's gland. The finding of PRP expression in von Ebner's gland, whose secretions have been suggested to play a role in taste stimulation, may add further support to a possible function of PRPs in bitter tasting. It is possible that the mRNA expression of statherin and histatin in macaque taste bud tissues (containing von Ebner's gland) may be related to statherin's regulation of salivary calcium, and histatins' antibacterial and antifungal properties.

Mouse Taste Genes: Identification, Chromosomal Locations, and Phenotypic Domains. DAVID B. HARDER (Dept. of Psychology, Florida State University, Tallahassee, FL 32306-1051)

Over the past two decades nine loci have been designated as having major effects on preferences for, and by implication gustatory sensitivity to, specific tastants. Each was named after the chemical compound for which preference or aversion differences among inbred strains of mice (*Mus domesticus*) were first noted. Segregation patterns within heterogeneous populations, from Mendelian crosses, and during development of congenic strains, plus strain distribution patterns in recombinant inbred sets, have since been adduced as supporting monogenic bases for the behavioral differences. Six loci affecting bitter aversion (*Soa*, *Rua*, *Qui*, *Glb*, *Cyx*, and *Ptc*) have been proposed. The first five have tentatively been located near each other on a portion of chromosome 6 homologous to human chromosome 12p. A site for *Ptc* has not been suggested. Three loci affecting sweet preference (*dpa*, *prs*, and *Sac*) have also been proposed. *Sac* has not been located, but the first two have been assigned to a portion of chromosome 4 homologous to human chromosome 9p. Evidence for the existence and location of the several loci varies considerably in amount and clarity. The degree to which behavioral effects of these "taste genes" reflect specifically gustatory differences, and the limits of those effects, have been variably characterized as well. *Soa* and *dpa*, the two most extensively studied, affect peripheral gustatory events, but the transduction processes involved are only beginning to be investigated.

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The Sucrose Octaacetate Taste Gene (Soa) is on Distal Mouse Chromosome 6 and is Closely Linked (or Identical) to Salivary Proline Rich Protein Genes (Prp). G. WHITNEY, C.G. CAPELESS, K.S. GANNON, D.B. HARDER (Psychology Dept, Florida State Univ., Tallahassee, FL 32306-1051), E.A. AZEN (Dept. Medical Genetics, Univ. of Wisconsin, Madison, WI), W. G. BEAMER, B.A. TAYLOR (The Jackson Laboratory, Bar Harbor, ME)

A chromosomal location for the gene influencing sucrose octaacetate avoidance in mice (Soa) was established by linkage analysis of behavioral and electrophoretic data from recombinant inbred (RI) strains, congenic strains, and test-cross progeny. Strain distribution patterns for Soa and Ldr-1, an electrophoretic marker locus on chromosome 6, were compared in the SWXL/Ty and SWXJ/Bm RI sets. Recombination was found in just 4 of 20 strains, yielding an estimate of 7.1 cM between the two loci. A similar distance was indicated by a set of replicate congenic strains. Seven of 11 B6.SW-Soa^a strains retained an Ldr-1 allele from the SWR/J donor of the Soa^a (taster) allele, for a distance of 5.5 cM. A comparable estimate, 10.0 cM, was obtained from the Soa / Ldr-1 recombination frequency in 90 backcross progeny of SWR/J and ABP/Le (nontaster) mice. A complete absence of recombination between Soa and Prp (electrophoretic haplotype) in the 20 RI strains, the 11 congenic strains, and in 10 SW.B6-Soa^b (nontaster) congenic strains still in development (generation 7 tested) suggested at least very close linkage (<0.7 cM) between these loci. On present evidence Prp and Soa could be identical. The region of chromosome 6 to which Soa and Prp map is homologous to a region of human chromosome 12p containing similar PRP genes.

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The Sucrose Octaacetate Taste Gene (Soa) Influences Response to Some Other Bitter Tastants. G. WHITNEY, J.C. MAGGIO, D.B. HARDER and J.D. BOUGHTER, JR. (Psychology Department, Florida State University, Tallahassee, FL 32306-1051).

The mouse autosomal gene Soa has a major effect on aversion to the bitter tastant sucrose octaacetate (SOA). The influence of the Soa locus on responsiveness to other compounds was investigated by comparing congenic strains which differ only for a short chromosome segment bearing the Soa locus. C57BL/6J inbred mice (B6) carry the Soa^b allele (SOA Taste Blind). The B6.SW-Soa^a mice are congenic with B6 but carry the Soa^a allele (SOA Aversion) which was transferred from the SWR/J inbred strain (SW) into the B6 line. The SW inbred donor strain was also tested. Eighteen tastants (60 concentrations) representing the classic taste qualities were evaluated. With the single exception of 10mM acetic acid, the Soa^a allele did not influence response to compounds which humans generally classify as sweet, sour, or salty. The Soa^a allele (or SW derived alleles at closely linked loci) did affect response to four of six bitter compounds (Denatonium Benzoate, Raffinose Undecaacetate, Strychnine Hydrochloride, Sucrose Octaacetate). Among bitters only Quinine (QSO4 & QHCl) did not reveal an Soa^a influence, possibly because the B6 inbred partner strain is already a sensitive quinine taster. The physiological mechanism affected by Soa polymorphism appears to mediate response to a structurally diverse array of bitter tastants.

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Pattern Analysis of SOA Drinking for B6.SW-Soa^a, C57BL/6J and SWR/J Mice. KIMBERLEY S. GANNON, JAMES C. SMITH and GLAYDE WHITNEY (The Florida State University).

Inbred strains of mice differ in their overall consumption of the bitter tastant sucrose octaacetate (SOA) as measured using two-bottle, 48-hr preference tests. At certain SOA concentrations, SWR/J mice drink very little (<0.5ml) of the substance and C57BL/6J consume equal amounts of SOA solution and distilled water. This strain difference is due to the influence of a major autosomal gene with two alleles, the "SOA-avoidance" allele being dominant to the "SOA-nonavoidance" allele. B6.SW-*Soa*^{a} congenic mice have been developed from C57BL/6J and SWR/J parental strains and possess the SWR/J "avoidance" allele on the C57BL/6J background genome (Whitney *et al., Behav. Genet. 19*:409, 1989). In order to conduct a fine-grained analysis of SOA drinking, B6.SW-*Soa*^{a}, C57BL/6J and SWR/J mice were tested using a computerized system which monitored licking behavior via electrical contacts. Detailed patterns of SOA vs water drinking were obtained for a descending SOA concentration series (10^{-3} to 10^{-8} M presented in log molar steps). Congenic mice were similar to SWR/J mice in their avoidance of the SOA solutions at all concentrations as determined by number of licks and total consumption. Frequently, an initial daytime sampling was sufficient to induce subsequent avoidance of the SOA-containing tube for the remainder of the 23-hr session. Evidence of other influences (possibly olfaction and/or learning) mediating SOA avoidance was found.

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Amino Acid Preference/Aversion Among Inbred Mice: Multivariate Implications for Genetic Architecture of Taste. C.G. CAPELESS, G. WHITNEY & D.B. HARDER. (Psychology Department, Florida State University, Tallahassee, FL 32306-1051).

Eleven amino acids (3 concentrations each) were tested by two-bottle preference procedures across 10 inbred strains of mice. Multivariate statistical techniques were used to investigate among-strain patterns indicative of genetic influences. Multi-Dimensional Scaling and Principle Components Analysis yielded configurations in three dimensions and three components (respectively) that were mutually consistent. The arrangement of four groups via several algorithms of Cluster Analysis was also consistent and interpretable within this common framework. The data were viewed as a tetrahedron in three dimensional space, the x, y, and z, axes aligned through the vertices and center of each face, all axes orthogonal. The four vertices of the solid were defined by clusters of substances centering about I) l-proline/d-alanine; II) d-tryptophan and the two isomers of valine; III) d-phenylalanine; IV) l-tryptophan/l-phenylalanine. Application of genetic correlation estimations indicated the I, II, and IV clusters as likely to contain large genetic components. Patterns for avoided amino acids were examined for possible influence of the postulated bitter taste genes Soa and Qui. Neither of these genes appeared to strongly influence the amino acid strain profiles. Ninomiya (1984, Brain Res. 322: 83-92; S. Roper & J. Atema (Eds) Olfact. & Taste IX: 527-529) has presented data suggesting major genetic influences on responsiveness to l-proline and d-phenylalanine which are consistent with the present model.

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Interspecific differences in bitter taste sensitivity influence whether mice eat monarch butterflies. JOHN I. GLENDINNING (Florida State University)

Three species of mice (*Peromyscus melanotis, P. aztecus* and *Reithrodontomys sumichrasti*) are abundant in the areas of Mexico where lipid-rich monarchs form overwintering aggregations, but only one, *P. melanotis*, eats the butterflies. In these aggregations, the monarchs are densely packed, cover the ground and foliage, and are unable to flee from nocturnal predators owing to low temperatures. However, the butterflies are not defenseless as their bodies contain bitter-tasting cardiac glycosides (CGs). Other species of mammals and birds have been shown to avoid foods treated with CGs. I hypothesized that *P. melanotis* is the only species tolerant to the bitter taste of the cardiac glycosides (CGs) in monarchs, and therefore is the only one that can eat monarchs. Two-choice preference tests were used to compare the taste sensitivity of all three species to digitoxin (a representative CG). Digitoxin is structurally similar to the CGs present in monarchs. Mice were offered ascending and descending series of concentrations of digitoxin. These concentrations were within the range of concentrations of CGs found naturally in monarchs. The avoidance threshold to digitoxin of *P. melanotis* was 38 times greater than that of *P. aztecus* and 720 times greater than that of *R. sumichrasti*. Based on these thresholds, and known concentrations of CGs in the butterflies, it appears that over 80% of the monarchs have suprathreshold concentrations of CGs for *P. aztecus* and *R. sumichrasti*, whereas less than 10% do for *P. melanotis*. Because CGs are absorbed extremely poorly across the gastrointestinal wall, it is unlikely that interspecific differences in toxicological sensitivities can explain these results. I conclude that *P. aztecus* and *R. sumichrasti* avoid eating monarchs because of an intolerance to the bitter taste of CGs.

MHC-Determined Odors are Present in Germ-Free Mice KUNIO YAMAZAKI, GARY K. BEAUCHAMP (Monell Chemical Senses Center, Phila., PA), LEWIS THOMAS (Cornell University Medical College, NY, NY) JUDITH BARD and EDWARD A. BOYSE (University of Arizona, Tucson, AZ).

The Major Histocompatibility Complex (MHC) of the mouse imparts to each mouse an odor that reflects its genetic constitution at this region of chromosome 17. Neither the chemical identity of the odor nor the pathway between genes and odor is understood. Hypotheses of odor determination include two major alternatives: i) the urinary odors of individuality may be due to small volatile fragments of the polymorphic MHC molecules themselves or to volatile secondary metabolites which are associated with the MHC molecules or fragments of these molecules; or ii) they may be due to odorous by-products of a commensal bacterial population, perhaps under immunological control by the MHC, or perhaps selectively bound to the distinctive MHC molecule prior to excretion in the urine. In order to test the latter hypothesis, urines were collected from C57BL/6 and congenic C57BL/6-H-2^{k} male mice born by Cesarean derivation and reared in a germ-free environment. The ability of trained mice to distinguish the urine odors of the germ-free mice in a Y-maze was investigated. The results demonstrated that mouse MHC odortypes are as readily produced in germ-free mice as they are in conventionally reared mice that possess the natural commensal bacteria populations. These data provide no support for the hypothesis that bacteria are involved in producing MHC-specific odors in mice.

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Genetic Control Over Salt Preference in Inbred Strains of Mice. GARY K. BEAUCHAMP (Monell Chemical Senses Center, Philadelphia, PA)

Inbred strains of mice are potentially powerful tools for investigating sensory control over ingestion of nutrients. Lush (personal communication) has found that several inbred strains of mice differ in their response to NaCl (salt) in long-term preference tests. A series of studies has been initiated to extend this preliminary work using C57BL/6J (B6) and 129 J (129) adult male mice.

In the first studies, standard 48-hr saline vs. water preference tests were conducted. All C57 mice rejected saline relative to water at all concentrations above.009 M. In contrast, 129 mice preferred salt to water at all concentrations except .300 M (the highest tested) and, puzzlingly, .038 M. In the next experiment, new groups of C57 and 129 male mice were obtained (n = 10 males/group), and the F_1 generation was bred from the animals used in the first experiment. Nineteen F_1 males, 10 offspring of C57 and 9 from the reverse cross, were tested. Three concentrations of NaCl solutions were used, 0.300 M, 0.075 M and 0.018 M. The order of presentation of the solutions during testing was completely counter-balanced. As before, for the 0.075 M concentration, there was a large difference between the two strains: all 129 mice exhibited a preference whereas the C57 mice did not. The F_1 animals were between the two inbred strains, being indifferent to the two lower concentrations and rejecting .300 M. Closer examination of the F_1 data indicated no maternal effects (i.e., offspring of C57 mothers were not different from offspring of 129 mothers). These data further support a genetic basis for the strain difference.

Next, the F_2 generation was bred from randomly selected F_1 parents. Preliminary data using only 0.075 M salt for testing indicated that approximately 1/4 of the animals strongly reject salt, behaving much like C57 mice. Although less clear, it also appeared that about 1/4 of animals resembled 129 mice in consistently preferring salt. A relatively simple genetic difference may underlie the observed strain difference in salt preference.

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Acute Olfactory Sensitivity and Specificity of Mature Male Goldfish to Water-borne Androgenic Steroids: A Class of Inhibitory Pheromones? PETER W. SORENSEN (Dept. of Fisheries & Wildlife, University of Minnesota, St.Paul, MN 55108), NORMAN E. STACEY (Dept. of Zoology, University of Alberta, Edmonton, Alberta T6G 2E9, Canada), and TOSHIAKI J. HARA (Freshwater Institute, Winnipeg, Manitoba R3T 2N6 Canada).

Several hours prior to ovulation and spawning female goldfish release the steroidal hormone 17α,20β-dihydroxy-4-pregnen-3-one (17α,20βP) to the water to function as a potent "priming" pheromone. The olfactory system of goldfish is acutely and specifically sensitive to 17α,20βP and within several minutes of exposure mature males experience 2-3 fold increases in gonadotropic hormone which in turn triggers increased milt (sperm) production. We now report that the stimulatory actions of 17α,20βP may be complimented by inhibitory actions of water-borne androgenic steroids. When androgens are added to the water they reduce 17α,20βP-induced milt increases in a dose-dependent manner. Furthermore, electro-olfactogram recording (EOG) has confirmed that the olfactory system of mature males detects water-borne androgens. Of 20 androgens tested, androstendione is the most potent olfactory stimulant having a detection threshold of 10^{-11}M. Although initial cross-adaptation experiments indicate a single, specific transduction mechanism, in vitro binding experiments suggest androgens could be functioning as antagonists for 17α,20βP receptors. Research is presently underway to resolve this question.

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Efflux and Uptake of Amino Acids by the Olfactory Organ of the Spiny Lobster: Effects on the Background Concentrations of Chemoexcitants in the Receptor Environment. H.G. TRAPIDO-ROSENTHAL, R.A. GLEESON, and W.E.S. CARR. (Whitney Laboratory, University of Florida, St. Augustine, Florida).

Amino acids such as taurine, glutamate, and glycine are odorants that activate specific populations of chemosensory neurons in olfactory sensilla of the spiny lobster, Panulirus argus. They are also present in the following high concentrations in the neurons and accessory cells of the sensilla themselves: taurine = 2 mM; glutamate = 20 mM; glycine = 100 mM. These sensillar cells also have specific uptake systems that can internalize amino acids against large concentration gradients. In spite of these uptake systems, there is a net efflux of excitatory amino acids out of sensillar cells into the receptor lymph. Using taurine as a model, we show that the taurine uptake system serves primarily as a "re-uptake" system that serves to maintain a low background concentration (or noise level) of this amino acid in the receptor environment by re-internalizing endogenous taurine leaking out of the cells of the olfactory sensilla. We estimate that, when the taurine uptake system is functioning, the background concentration of taurine in the receptor environment can be as high as 50 and 100 nM. In the presence of guanidinoethane sulfonate, a potent and selective inhibitor of taurine uptake, the background concentration can be increased 20-fold. Effects of background concentration on the sensitivity of receptor cells to exogenous amino acids are described in the accompanying presentation by Gleeson et al.

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Leakage and Reuptake of Intracellular Amino Acids: Implications for Olfactory Cell Sensitivity. R.A. GLEESON, H.G. TRAPIDO-ROSENTHAL and W.E.S. CARR (The Whitney Laboratory, University of Florida)

In marine invertebrates certain intracellular free amino acids are found at high concentrations (millimolar) and are known to be significant osmotic effectors important in the maintenance of cell volume and preservation of macromolecular structure and function. The particular amino acids present varies among species and even among tissues; however, those commonly involved include taurine, glutamate, alanine, glycine, proline and aspartate (Clark, 1985. In: Transport Processes, Iono- and Osmoregulation). Many of these very same amino acids are known to be potent excitants and/or suppressants of lobster chemoreceptors that have threshold sensitivities within the nanomolar to micromolar range. To better understand how chemoreceptor sensitivity is maintained in the face of high levels of intracellular amino acids, we have measured the intracellular concentrations of selected amino acids in the olfactory sensilla of the lobster, Panulirus argus, and have examined the relationship between net efflux ("leakage") and uptake (see poster by Trapido-Rosenthal et al.). Based on these biochemical measures, we have physiologically evaluated how the sensitivities of chemoreceptor cells are altered in the absence of uptake processes. The results suggest that the reuptake of amino acids leaking from intracellular compartments is an important mechanism for maintaining chemoreceptor sensitivity.

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Response Reliability of chemoreceptor cells: influence of backgrounds and mixtures. JENNIFER CASTERLINE, CARL MERRILL, RAINER VOIGT, and JELLE ATEMA (B.U.M.P., M.B.L., Woods Hole, MA 02543, USA)

Mean response magnitudes of even small populations of hydroxy-L-proline (Hyp) sensitive chemoreceptor cells of the lateral antennule of *Homarus americanus* are reliably reproduced with repetition of stimulation. However, individual cell output is far less reliable. Our previous studies of reliability excluded influence of modulation by compounds other than Hyp by testing with "pure" Hyp solutions in artificial seawater (ASW). The present study determines response reliability under more natural conditions. In the first part of the study, the sensitivity of cells to hyp was measured at one log-step intervals over a range of 10^{-8} to 10^{-4} M Hyp and then tested with five repetitions of either 10^{-4} or 10^{-5} M Hyp depending on its sensitivity, and a single presentation of a seawater extract of mussel flesh with undetermined Hyp concentration (mussel extract). These steps were repeated in the following sequence of backgrounds: ASW, raw seawater (RSW), ASW, an equimolar mixture of 14 compounds excluding Hyp (14C), and ASW. In the second part, Hyp was presented as a component of an equimolar mixture of glycine and Hyp, a 15 compound equimolar mixture (14C plus Hyp) and within a mussel extract. Responses of chemoreceptors to 10^{-6}, 10^{-5} and 10^{-4} M Hyp, 14C (all compounds at 10^{-4}M), and 10^{-4} M glycine were determined. Then each cell was tested with five repetitions of each of four potential stimuli: Hyp, glycine-Hyp mixture, 14C plus Hyp, and mussel extract. The results showed that individual cell responses in general were less reliable than population means. Neither the stimulus response functions nor the series of repetitions for the responses to Hyp were affected by RSW. The 14C background partially cross-adaptated the cells, reducing response magnitude. The different backgrounds did not affect the responses to mussel extract. In some cells, responses to Hyp in one or more mixtures were more reliable than response to Hyp alone. Individual cells varied widely in terms of their realtive sensitivity to the mixtures and to Hyp alone. Response reliability of receptor cell populations is behaviorally more relevant when stimuli are presented as mixtures in complex chemical backgrounds than when presented as single compounds in ASW. Supported by NSF (BNS 88-12952) to JA.

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Boundary layers and microscale fluid dynamics around chemosensory appendages. PAUL A. MOORE, and JELLE ATEMA (Boston Univeristy Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

Any chemosensory structure within a flow field, such as a tongue, antenna, or receptor epithelium, has a boundary layer surrounding it. It is in this region that the turbulence-dominated odor dispersal slowly gives way to diffusion-dominated odor dispersal. This boundary layer modifies the temporal structure of odor signals passing to the receptor cell surface. To study the filtering effect of boundary layers on odor signal structure, we used the olfactory organ of the lobster, *H. americanus*. The lateral antennules of *H. americanus* contain a dense tuft of aesthetasc sensilla, creating a large boundary layer around the sensilla, which effectively prevents flow from entering. Without flow odor access is very slow. When the antennule flicks, the sensilla are forced through the water at high velocity decreasing the boundary layer thickness and allowing rapid odor access. To quantify these effects we placed microelectrochemical electrodes within the aesthetasc hairs of the lateral antennule under flicking and non-flicking conditions. Calibrated odor pulses were delivered to the antennule under controlled flow conditions. These pulses were sampled at the probe surface at 10 and 200 Hz. Stimulus filtering by boundary layers plays an important role in determining the temporal profiles of odor pulses available to receptor cells. In an evolutionary sense, boundary layers may have influenced receptor adaptation and disadaptation time constants.

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Effects of Stimulus and Background Concentration on Cumulative Adaptation in Chemoreceptor Cells. RAINER VOIGT and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543, USA)

The lateral antennules of the lobster, *Homarus americanus*, play an important role in olfactory orientation. Taurine (Tau) sensitive chemoreceptor cells on the antennules form a largely narrowly tuned and highly sensitive cell population. Tau occurs in high concentrations in lobster prey and in extremely low concentrations in costal waters; this low natural background may enhance the value of Tau as a long distance orientation cue. Natural odor plumes can be described by stimulus pulse intensity, background concentration and repetition rate of pulses. To determine the adaptation and dis-adaptation properties of chemoreceptor cells we tested single chemoreceptors with a series of 10 Tau pulses in different concentrations and different background concentrations and varied the interstimulus intervals. Chemoreceptor cells were recorded extracellulary with suction electrodes. Single cells were identified with a standard Tau pulse ($7x10^{-5}$ M). A train of 10 pulses in one of four concentrations ($7x10^{-4}$ M to $7x10^{-7}$ M) was applied in a 10^{-7} M Tau background in one of three pulse intervals (2.5 s, 5 s, 10 s).Each of these 12 trains was separated by a 3min recovery period. After 3 min of recovery in artificial sea water the series of trains was repeated in 10^{-6} M, 10^{-5} M, 10^{-4} M Tau backgrounds. Individual cells showed a wide range of cumulative adaptation. Stimulus-response functions revealed range fractionation. In general, shorter pulse intervals resulted in gradually stronger cumulative adaptation . Weaker pulse concentration caused less cumulative adaptation. All but the highest background had negligible effects on response magnitude showing the efficiency of background adaptation. Cumulative adaptation occurred mostly during the first three stimuli and mostly with strong pulse concentration in low background. Thus, low-firing cells showed good response reproducibility (i.e. no cumulative adaptation) even with the shortest pulse interval, whether low firing rates were caused by internal cell properties, low stimulus concentration or high background concentration. This state of adaptation may be natural for cells operating in odor plume conditions.

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Spectral tuning of lobster olfactory cells and their response to defined mixtures and natural food extracts. Anna Weinstein, Rainer Voigt, and Jelle Atema (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543, USA)

Lobster antennules are chemoreceptor organs that play a major role in orientation behavior. Hydroxyproline-(Hyp) and Taurine-(Tau) best cell poulations dominate their amino acid tuning spectrum. We tested the response of chemoreceptor cells on the lateral antennule to fifteen amino acids and to six mixtures, three containing the amino acids in known concentrations, and three natural extracts. The artificial mixtures we used reflect the prominence of Hyp and Tau as best compounds for antennular chemoreceptors. All three mixtures had a total concentration of 1.5×10^{-3} M and contained Hyp in equal concentrations. "Tau-Hyp mixture" had equal amounts of Tau and Hyp, "Tau enhanced mixture" had three times as much Tau than Hyp, and "equimolar mixture" had all the compounds in equal concentration. The natural mixtures were derived from fresh crab, fish, and mussel flesh. Searching with the Tau-enhanced mixture revealed cell populations of predominantly Hyp, Tau, and glutamate best cells, a distribution similar to that found by searching with equimolar mixture (Johnson & Atema 1983, Neuroscience Letters 41: 145-150).
Mixture response was generally suppressed relative to best compound response; the degree of suppression was usually independent of a cell's tuning breadth. Most Tau-best cells, particularly narrowly tuned Tau cells, showed enhanced response to the Tau mixture relative to the other artificial mixtures. The range of response to the natural extracts (0-375 spikes) greatly exceeded the range of response to the mixtures (0-72 spikes), although average response over all cells to all mixtures and extracts was similar. Two major "mixture populations" exist; one shows glutamate and Hyp cells with enhanced responses to the artificial mixtures relative to the natural extracts, and the other shows mainly Tau cells with stronger responses to natural extracts than to the mixtures.

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Olfactory receptors in Arctic char (*Salvelinus alpinus*) with high sensitivity and specificity for prostaglandin $F_{2\alpha}$. TORARINN SVEINSSON and TOSHIAKI J. HARA (Department of Zoology, University of Manitoba, Winnipeg, Manitoba, R3T 2N2, and Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Manitoba, R3T 2N6, Canada)

Reproductively active Arctic char release F-series prostaglandins (PGs) into the surrounding water where they possibly function as reproductive pheromones. To test whether Arctic char has olfactory recpeptors for these PGs we examined stimulatory effectiveness of some commercially available prostaglandins, using electroolfactogram (EOG). Of the 12 PGs tested, 6 (Dimethyl-$PGF_{2\alpha}$, $PGF_{2\alpha}$, $PGF_{1\alpha}$, $PGF_{2\beta}$, $PGF_{3\alpha}$, 11β-$PGF_{2\alpha}$, in descending order) gave responses at concentrations below 10^{-8} M. The threshold value for the most stimulatory compounds, $PGF2\alpha$, and its synthetic analog dimethyl-$PGF_{2\alpha}$, was 10^{-11} M. The concentration-response curve on a semilog graph showed a typical sigmoidal shape which saturated around $5 \cdot 10^{-8}$ M, suggesting involvement of a single type of receptor. The results demonstrated a high stereospecificity of the receptors responsible for these responses. Alteration of the chemical bond of the hydroxy group (-OH) from down to upward direction at the carbon number 9 (9β-$PGF_{2\alpha}$), 11 (11β-$PGF_{2\alpha}$), or 15 (15(R)-$PGF_{2\alpha}$), reduced the affinity of the receptor for the compound by more than 2 log units. High species specificity was also indicated. Experiments on lake char (Salvelinus namaycush) showed a similar specificity and sensitivity for prostaglandins as found in Arctic char. However, none of the prostaglandins tested were effective in rainbow trout (Oncorhynchus mykiss) and brook char (Salvelinus fontinalis) at 10^{-6} M.
Currently we are developing a binding assay for biochemical identification and characterization of this PGs receptor from the olfactory epithelium in Arctic char, using tritiated $PGF_{2\alpha}$ as ligand.

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Olfactory receptor neurons from developing male Manduca sexta antennae respond to species-specific sex pheromone in vitro

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Males of the moth Manduca sexta detect species-specific sex pheromone released by female moths by means of male-specific olfactory receptor neurons (ORNs). To investigate primary sensory transduction mechanisms, antennae from stage-3 male Manduca sexta pupae were dispersed into single cells and kept in long-term primary cultures. In vitro the olfactory receptor neurons (ORNs) could be identified with two monoclonal antibodies, one of which specifically recognizes adult, male-specific pheromone-sensitive ORNs in situ (Hishinuma et al., 1988; Stengl and Hildebrand, 1988). Patch-clamp studies demonstrated that cultured ORNs express at least three different kinds of Cs^+-blockable K^+ channels and one type of TTX-blockable Na^+ channel after 3 weeks in vitro (Stengl et al., 1989). After ≥3 weeks in vitro, male-specific ORNs respond to DMSO extracts of female pheromone glands, or to the pheromone component bombykal with large inward currents. After washes with extracellular solutions, single channel openings could be resolved in the inward current: an unspecific cation channel and the previously described Ca^{2+}-dependent K^+ channel (with 150 mM KCl in the pipette to mimic the receptor lymph). Also, the previously described, ATP- and cGMP-blockable delayed outward rectifier channel changed its open-time probability. After prolonged exposure to the pheromone, all channels closed, and after addition of presumptive pheromone-binding protein or BSA, channels reopened. We now seek to learn whether these responses underlie the depolarizing receptor potentials in the male-specific olfactory receptor cells.

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Odor-activated K^+ conductance inhibits lobster olfactory receptor cells. W. C. MICHEL and B. W. ACHE. Whitney Lab. and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

This study extends our recent finding that some odors reduce the magnitude of odor-evoked depolarizations in lobster olfactory receptor cells by activating an outward (hyperpolarizing) current (Chem. Sens. 14:637). All experiments were conducted on current-clamped neurons recorded *in situ* and stimulated by an olfactometer that simulates the rapid sampling of the animal (flicking). Hyperpolarizations rose less rapidly than did depolarizations of equal magnitude. The average onset slope of hyperpolarizations ($X=-0.04$ mV/msec, $n=27$) was significantly less (t-test; $p<0.001$; comparison of absolute values of slopes) than that of depolarizations (0.17mV/msec, $n=41$). These differences in slope presumably reflect differences in the underlying ionic conductances. In contrast to the inward currents, which appeared to be carried by Na^+ and/or Ca^{+2}, the outward currents appeared to be carried by K^+. When the K^+ gradient was increased by reducing extracellular K^+ from 14 to 1.4 mM, the magnitude of odor-evoked hyperpolarizations reversibly increased to 137% ($n=10$) of the control level. Further, 10 mM cesium, as well as 10 mM 4-aminopyridine, reduced the amplitude of odor-evoked hyperpolarizations to 68% ($n=3$) and 28% ($n=6$) of control levels, respectively. Co-activation of a K^+ conductance would be expected to slow the onset of depolarizing responses. Indeed, the rate of depolarization evoked by a complex mixture (TetraMarin extract) in cells known to contain hyperpolarizing conductances was significantly less than that in cells for which no hyperpolarizing conductances could be identified (0.13 vs 0.21 mV/msec; $n=19$ & 22; t-test; $p<0.05$). The slowed rate of depolarization was sufficient to delay the time to threshold by a calculated average of 50 ms. Thus, components of odor mixtures that inhibit lobster olfactory receptor cells do so by activating a K^+ conductance that both slows the rate and decreases the magnitude of the net depolarization.

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Excitatory and Inhibitory Responses Induced by Amino Acids in Isolated Mudpuppy Olfactory Receptor Neurons. VINCENT E. DIONNE (University of California, San Diego)

Viable receptor neurons were dissociated without enzymes from mudpuppy olfactory epithelia and their responses to chemical stimuli studied with patch recording methods. The neurons were isolated from small pieces of tissue by exposure to low-Ca saline at pH 10.3 (mM: 90 NaCl, 4 NaOH, 3 KCl, 1 $CaCl_2$, 10 Na_2CO_3, 10 $NaHCO_3$, 10 $Na_2C_4H_4O_4$, 1% BSA) followed by gentle trituration. Electrical activity was monitored with a patch-clamp using the tight-seal, on-cell configuration. This allowed sensitive detection of action potentials from the extracellular currents they induced. The cells were stimulated in a flowing saline bath with amino acids applied by pressure from blunt-tipped perfusion pipets. In separate experiments amino acids were shown to be effective olfactory stimuli in these animals by recording the electro-olfactogram *in situ*. The response from single receptor neurons stimulated by amino acids was a change in the frequency of action potentials. In the absence of added amino acids, responsive cells exhibited spontaneous action potentials at 1-2 Hz. Application of amino acids caused some cells to increase their frequency of action potentials up to 20-fold. In contrast the same stimuli caused other cells to cease firing. These changes in action potential frequency were reversible. Perfusion with saline alone did not cause responses from sensitive cells, and many cells with spontaneous action potentials did not respond to the amino acids that were tested. These results suggest that the olfactory system may transduce and encode chemosensory information with both ON and OFF responsive units.

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The Role of Cyclic AMP as a Second Messenger in Vertebrate Olfactory Transduction. STUART FIRESTEIN and GORDON M. SHEPHERD (Yale University Medical School)

Cyclic AMP was first implicated in the olfactory response by biochemical assays on homogenates of cilia membrane from whole epithelia. In single olfactory cells a cAMP gated conductance has been described, but it remains to demonstrate that cAMP is specifically a modulator of the odor response in individual responding receptor neurons. We have recorded the odor induced current in isolated salamander olfactory receptors and utilized standard pharmacological treatments in order to demonstrate an essential role for cAMP in olfactory transduction. IBMX, a phosphodiesterase inhibitor, prolongs the odor elicited current by several seconds in a reversible manner. Likewise GTP- -s, which uncouples G-proteins from receptors, and Forskolin, an adenylate cyclase agonist, also prolong the odor response. GDP- -s, an inhibitor of G-protein activity, blocks the odor current in a time and stimulus dependent manner. Chlorophenyl-thio cAMP (CPTcAMP), a membrane permeable cAMP analogue, induces a current which is electrically identical to the odor induced current and which occludes the odor induced current when cells are stimulated first by CPTcAMP and then by odors. These results provide a final link between the biochemical and electrophysiological evidence for the involvement of cAMP as a second messenger in olfactory transduction.

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Purification and characterization of odorant binding proteins from nasal mucosa of pig and rabbit. P. PELOSI and M. DAL MONTE (Istituto di Industrie Agrarie, University of Pisa, Pisa, Italy).

Two binding proteins for 2-isobutyl-3-methoxy-pyrazine have been purified to homogeneity from the nasal mucosa of the pig and the rabbit respectively. They are very abundant, accounting for about 1% of total soluble proteins and have been obtained as single bands on SDS electrophoresis after repeated chromatography of the crude extracts on ion exchange columns. On the basis of the molecular weights measured under denaturing conditions and by gel filtration, both proteins appear to be homodimers. The subunits' molecular weights are 22,000 for the pig and 18,000 for the rabbit. Their isoelectric points, as determined by isoelectric focusing in a gradient of Ampholines 3.5-9.5 are 4.2 and 4.7 respectively. Tritiated 2-isobutyl-3-methoxy-pyrazine binds with dissociation constants in the range of 0.1 µM to both proteins. On the basis of these data, the rabbit protein appears very similar to those already purified from cow and rat, while the porcine one seems a little different both in terms of molecular weight and isoelectric point. Preliminary data on their amino acid composition support such similarity with the two known proteins and suggest similar physiological functions. Antibodies are currently being made for investigating their anatomical localization.

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Whole Cell Patch Recordings Show Frog and Salamander Olfactory Receptor Neurons are Different. RAYMUND PUN and ROBERT C. GESTELAND (Departments of Physiology & Biophysics and Anatomy & Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267).

We are comparing cellular mechanisms mediating excitability in olfactory receptor neurons (ORNs) of the grass frog, *Rana pipiens*, and the land phase tiger salamander, *Ambystoma tigrinum*. In particular we are studying the properties of the fast inactivating inward current, probably the Na^+ channels. Our experiments suggest that the membrane electrical properties of ORNs from the two invertebrates are dramatically different.

ORNs from the frog and the salamander were isolated from the nasal epithelia by gentle scraping of the dissected epithelial surface. Cells were are then suspended in a HEPES-buffered amphibian saline (pH 7.3-7.4; 240 mOs). Whole-cell "tight seal" recording was performed on morphologically identifiable ORNs. Recording-pipette solution was a HEPES-buffered high-K^+ solution (pH 7.1; 225 mOs).

ORNs of the frog had a membrane potential of -48 mV, an input resistance of 1-2 GΩ and a capacitance of 4-5 pF. At resting membrane potential, action potentials (APs) could not be elicited unless the membrane potential was hyperpolarized by injection of current. However, long-duration action potentials which may continue for hundreds of ms could be generated following short-duration hyperpolarizing pulses. Spontaneous activity was not observed. Inactivation of the fast inward currents was voltage dependent with a $V_{1/2}$ of -90 mV. In contrast, ORNs of the salamander had a membrane potential of -60 mV, an input resistance of 0.7 GΩ, and a capacitance of 10-11 pF. APs were readily elicited at resting membrane potential and, in some cells, spontaneous activity occurred. Inactivation of the fast inward current was also voltage dependent but with a $V_{1/2}$ of -54 mV, which is more positive than that obtained for the frog.

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Mixture Interactions towards Binary Odorant Mixtures in Spiny Lobster: Electrophysiological Assay using Single Antennular Olfactory Receptor Cells. CHARLES D. DERBY, MARIE-NADIA GIRARDOT, AND PETER C. DANIEL (Dept. of Biology, Georgia State University, Atlanta, GA)

We are investigating mechanisms responsible for the detection and discrimination of binary mixtures and their components. In this study, we have examined the responses of 98 single antennular olfactory receptor cells to AMP, betaine, cysteine, glutamate, NH4, succinate, taurine, and all possible binary mixtures. For each cell, the following attributes were determined: response specificity; D-R curve for the best stimulus; D-R curves for the best stimulus + each of the other compounds at 5mM. Measured responses were statistically compared with predicted responses for each cell and for the population of cells. The substitution model assumes that a cell possesses one receptor type for all compounds; the polynomial model assumes a cell possesses a different receptor type for each compond. To identify mixture interactions, we have used a conservative approach: only those measured responses lying outside the range of values established by the predictions of the substitution model and the polynomial model are said to be cases of mixture interactions. Based on statistical criteria, mixture suppression is common, either when cells are analyzed individually or as a population. Mixture suppression is especially but not exclusively prevalent when one of the components of the mixture is cysteine, glutamate, or AMP. We are presently comparing the measured responses to the predicted responses from a "mixed" model, which more accurately models the actual receptor complement in the antennular receptor cell population, and comparing these electrophysiological results with behavioral results (see Daniel and Derby abstract).

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The spatial distribution of olfactory receptor currents. GRAEME LOWE and GEOFFREY H. GOLD (Monell Chem. Senses Ctr., Philadelphia, PA).

We have investigated the spatial distribution of the odor-induced conductance and resting K^+ conductance of tiger salamander olfactory receptor cells. The current across the somatic membranes of dissociated receptor cells was measured with a suction electrode, while locally stimulating the cells with a solution containing odorants and/or high K^+. Continuous flow of Ringer's solution, from the cell body toward the cilia, was used to orient the dendrite and cilia along a single axis, and to limit the region of the cell exposed to the stimulus. The high K^+ and odorants both induced outward currents across the somatic membrane; these outward currents are presumably caused by the depolarization of the somatic K^+ conductance by the (inward) generator current. Maximal odor-induced currents were ca. 80 pA. This is smaller than the generator current measured under voltage clamp (e. g., Firestein and Werblin, 1988), since the odor-induced depolarization must reduce the driving force for the generator current.

Odor sensitivity is restricted to the ciliary region. Starting at the tips of the cilia, response amplitude increased approximately linearly with the length of cilia exposed to the odor stimulus, reaching a maximum at the bases of the cilia. Thus, all portions of the cilia make equal contributions to the somatic currents. This indicates that the electrotonic length constant of the cilia is at least several times longer than their physical length. The latency and timecourse of the odor response was independent of the position of the stimulus along the cilia. Thus, transduction is a local process, and does not require the diffusion of odorants or intracellular messengers from the cilia to the dendritic membrane. The resting K^+ conductance of these cells was found almost exclusively in the cell body, i. e., extracellular K^+ stimulation had no effect when applied to the cilia or dendrite. This localization of the K^+ conductance should maximize the spread of current from the cilia to the cell body.

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Temporal Characteristics in Mucosal Inherent Activity Patterns: Evidence from Voltage-Sensitive Dyes. P.F. KENT, M.M. MOZELL, and S.J. MURPHY (Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, NY 13210)

We earlier demonstrated that the fluorescent dye WW781, could monitor the same mucosal events in response to odorants as does the EOG. In a more fully developed investigation we recently monitored the fluorescent changes in the dye WW781 at 100 contiguous sites in a 10 x 10 pixel array on the bullfrog and salamander olfactory mucosas every 10 ms in response to odorous stimuli. The odorants were d-limonene, butanol, and amyl acetate, each presented at two concentrations in a 3:1 ratio. Each odorant was humidified and puffed uniformly upon the entire mucosa for a duration of .7s at a rate of 300 cc/min. We recorded these spatial activity patterns on the floor of the salamander's olfactory sac (6 salamanders), on the floor (6 frogs) and the roof (6 frogs) of the bullfrog's olfactory sac. In addition to demonstrating different spatial activity patterns based upon response magnitudes for different odorants, we now report different spatial activity patterns based upon temporal characteristics. That is, each odorant produced a different pixel by pixel pattern for the times at which the responses started and ended. These combined spatial and temporal patterns are best visualized using a video format. For any given odorant, the temporal patterns paralleled the patterns given by response magnitudes. In general, the larger the magnitude of the response, the shorter was the start time and the longer the time till the response ended. This raises another possible dimension for the encoding of odorants, viz. an inherent spatially distributed temporal pattern which parallels the spatially distributed inherent magnitude patterns. Perhaps it is now necessary to further consider how the encoding of odorants at the level of the olfactory bulb might be affected if the discharges from different mucosal regions arrive and end at different times.

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Different Odorants Give Different Flow Rate Effects on The Magnitude of The Olfactory Response M.M. MOZELL, P.F. KENT, and S.J. MURPHY (Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, NY 13210)

In an earlier study using octane as the odorant, we found that the magnitude of the summated multiunit discharge recorded from the bullfrog olfactory nerve decreased with flow rate. This is opposite to what other studies would have predicted. However, most of these other studies may have confounded the effect of flow rate with the strong effect of number of molecules, which was allowed to vary with flow rate. In a later study using four odorants the effect of flow rate appeared to be either positive or negative depending upon the strength of the particular odorant's sorption to the mucosa. To pursue this more systematically, we recorded the summated multiunit discharges from two points on the bullfrog's olfactory nerve supplying widely separated loci along the mucosal flow path. Precise artificially produced sniffs were drawn into the intact olfactory sac either in the forward direction (6 frogs) or in the reverse direction (6 frogs). To vary flow rate while keeping the number of molecules and concentration constant, we varied sniff duration. The flow rates (in the bullfrog's normal range) were for each odorant 40, 70, 130, 223 ml/min. The odorants (octane, d-limonene, amyl acetate, geraniol, benzaldehyde and carvone) represented a wide range of mucosal sorption strengths. The results confirm that flow rate has differential effects upon the discharge magnitude of the olfactory nerve. For strongly sorbed odorants response magnitude grows with flow rate, but for weakly sorbed odorants this becomes an inverse relationship. Perhaps for the poorly sorbed odorants the increased flow rate across the mucosa reduces even further the probability of any molecule's sorption. Highly sorbed odorants, which at lower flow rates have the bulk of their molecules sorbed early in the flow path, might, at higher flow rates, present more molecules farther downstream. At any rate, we should now reconsider the generally held view that the magnitude of the olfactory response increases with increasing flow rate; apparently, the effect of flow rate can be positive or negative depending upon the particular odorant presented.

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Presenting Author: Essie Meisami

Olfactory Cavities of Rodents: Novel Problems Arising from Unique Features. ESSIE MEISAMI (Dept. of Physiology, Univ. of Illinois, Urbana IL 61801)

The rat olfactory epithelium is extensive, over 3 sq cm on each side of the nasal cavity. Careful morphometry shows that 90% of this area occurs within a completely enclosed "olfactory sac" located superior and posterior to the nasal passages proper. The sac's maximum dimensions are: length 10 mm, width 5 mm, height 5 mm. The sac's inlet is located anteriorly, at the beginning and above the nasopharyngeal duct. The thin (0.1 mm) floor of the sac prevents air movement between it and the underlying nasopharyngeal duct. In contrast to fish, amphibians and primates, the rat olfactory sac appears to have no true outlet, being essentially a cul-de-sac. The sac's walls becomes extensively folded during development, creating several conchae which expand the olfactory surface area and create several smaller and narrower passageways some of which are also cul-de-sacs. Knowledge on airflow pattern and spatial distribution of odorants in nasal passages is mainly based on the human and frog. Little is known about the dynamic of airflow through the rodent type of cavity; equally important is how air leaves these cul-de-sacs. Another feature of the rodent naso-olfactory cavities, not present in frogs and humans, is the existence of a "septal window" that permits air exchange between the right and left nasal passageways. This window, about 2 mm in length and 1 mm in height, occurs ventrally in the septum, a few mm posterior to the vomeronasal organ and is created because of septum's incomplete contact with the nasal floor. At the sides of the window, the sac's floor is open, allowing air passage between right and left nasal cavities. The septal window is already present at birth. If rodents are capable of alternating their breathing between the left and right side, the septal window may provide a way through which air can exit from the blind olfactory sacs.

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Taste Loss Due to Herpes Zoster Oticus: An Update after 19 months. CARL PFAFFMANN (Rockefeller University), LINDA M. BARTOSHUK (Yale University).

A case (C.P.) of Herpes zoster oticus, or Ramsey-Hunt's syndrome, presented with unilateral (left) auditory, facial and glossopharyngeal nerve damage. The viral infection began in late April 1988. We presented psychophysical measurements of the initial recovery process one year ago (AChemS, 1989). We now provide additional measurements showing recovery over 19 months. Taste function was measured with a spatial Q-tip test developed for the Connecticut Chemosensory Clinical Research Center (CCCRC). Stimuli (1.0 M NaCl, 1.0 M sucrose, .032 M citric acid, .001 M quinine hydrochloride) are "painted" on localized regions of the left and right sides of the front of the tongue (innervated by the chorda tympani nerve), the rear edges of the tongue (innervated by the glossophyaryngeal nerve), and the palate (innervated by the greater superficial petrosal nerve). In addition, a small quantity of each solution is tasted with the whole mouth and swallowed. The intensity of the taste sensations is judged on a 9-point scale (1=very weak, 5=medium, 9=very strong). Initially, no tastes were perceived on the left side; however, whole mouth function was essentially normal and C.P. did not experience any "real world" taste loss. The rear edge of the tongue and the palate began to show recovery about 4 months after the infection began. As taste intensities grew on the left rear of the tongue and the palate, they diminished on the right on all loci including the front of the tongue. This suggests that the left side may be exerting inhibition on the right. The recovery on the left front of the tongue is of special interest. This area took the longest to show signs of recovery (about 15 months after the infection). In addition, the ability to taste sweet recovered first and was the sole quality perceived for about 4 months.

We thank NIH grants DC 00283 and DC00168.

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Olfactory Responses to Enantiomers. B.J. COWART (Monell Chemical Senses Center)*

Several investigators have reported that many humans can differentiate the odor qualities of carvone enantiomers. Recently, it was also noted that (+)-carvone [+CAR] elicits more intense odor sensations than (-)-carvone [-CAR] at equivalent concentrations (Pike et al., Chem. Senses, 1988). Since discrimination studies have either failed to take that difference into account or made average intensity matches, which may not be appropriate for any given subject, successful discrimination performance could have reflected intensity rather than quality differences. We confounded intensity cues in a triangle test of discrimination (the odd stimulus in each triad matched one of the others in concentration; the similar stimuli differed in concentration). The same Ss (N=12) were also tested for threshold sensitivity to the two carvones and rated the intensity of suprathreshold concentrations of each; their responses to (+)- and (-)-fenchone were assessed in the same manner. There were no differences in thresholds or intensity ratings for the two fenchones, and only 2 Ss achieved criterion performance (7/12) in the triangle test. In contrast, thresholds for -CAR were significantly lower than those for +CAR, whereas suprathreshold +CAR was perceived to be more intense than suprathreshold -CAR. Five Ss exceeded criterion discrimination performance (9-12/12). Thus, there were a number of differences between the carvone enantiomers in their sensory properties, although quality discrimination under these conditions was less common than has been reported. Finally, discrimination performance was significantly related to the magnitude of the difference Ss perceived between the intensities of +CAR and -CAR (Spearman's rho = 0.76).

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Qualitative and Quantitative Responses of Osmic and Anosmic Subjects to Threshold Concentrations of Various Odors. DAVID A. STEVENS (Clark University, Worcester, MA 01610), and ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, USA)[1]

Individuals with specific anosmias are generally defined as those who seem to have a good sense of smell but lack the ability to perceive the aroma characteristic of a particular odorant. In anosmics, this deficiency is usually expressed by an elevated threshold and a pronounced shift in the perceived odor quality. The diastereoisomeric ketone, *cis*-4-(4'-t-Butylcyclo-hexyl)-4-methyl-2-pentanone (pemenone), shares with 5α-androst-16-en-3-one (androstenone) a pronounced urine-sweaty type odor. We previously determined intensity ratings and quality reports for suprathreshold concentrations of these compounds and several other materials described as urinous or which were said to exhibit specific anosmias (O'Connell *et al., Chemical Senses* 14:293-302, 1989). A principal-component analysis of these data revealed a significant relationship between the intensity scores for pemenone, androstenone and several of the other odorants and a corresponding clustering of the odor descriptors used to characterize these materials by subjects judged to be anosmic for the urinous note. Here, pemenone thresholds were determined for several hundred human subjects and a matched set of 62 anosmic and osmic subjects were selected for further study. Thresholds were determined for all of the odorants under consideration and the quality reports generated by threshold concentrations of each were elicited. Both principal-component and multiple regression analyses were performed. When the thresholds and quality reports of subjects, first characterized by their pemenone threshold were compared a number of significant interactions were observed. A subject's threshold and quality report for pemenone was significantly correlated with the threshold and quality report elicited by androstenone. These, and a number of other findings which collectively provide clues about the defects which give rise to specific anosmias are described.

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Clinical Diagnosis and Treatment of Olfactory Dysfunction: Sensorineural vs. Conductive disorders. PETER G. HEYWOOD, ROBERT J. DELORENZO, WILLIAM W. CAMPBELL, ARISTIDES SISMANIS, AND RICHARD M. COSTANZO (Departments of Neurology, Otolaryngology, and Physiology, Medical College of Virginia, Richmond, Virginia, 23298).

We report the preliminary findings from a study of 133 patients evaluated at the MCV Smell and Taste Clinic during its first year of operation. Patients were categorized according to their presenting diagnosis; head injury (42), post-viral anosmia (23), nasal congestion (19), idiopathic (13), Alzheimer-type dementia (8), nasal polyps (7), and other (21). All patients were administered a complete olfactory function evaluation including a detection threshold and odor discrimination test. Clinical interviews were conducted of all patients to establish a medical history and onset of smell loss. A sensorineural basis for olfactory dysfunction was established in 65 patients. The majority of these patients suffered head injury, Alzheimer-type dementia, or stroke and were not amenable to therapy. In several cases of idiopathic smell loss, olfactory function testing identified cortical neuropathy which was later verified with computerized tomography. The remaining 68 clinic patients received nasal endoscopy to determine if a conductive etiology was responsible for their olfactory disturbance. A number of conductive disorders were identified including nasal polyps, rhinitis, and septal deviation. Approximately one third of all clinic patients were diagnosed as suffering a conductive olfactory disturbance. These patients were administered topical nasal steroids, antihistamines; or underwent polypectomy, cauterization of the turbinates, or septoplasty. Follow up olfactory tests were administered and preliminary results indicate nasal steroids and polypectomies were most often associated with a complete recovery of olfactory sensation. Olfactory function testing is an important component for differential diagnosis and treatment of sensorineural and conductive olfactory disorders.

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Bilateral Olfactory Dysfunction in Early Stage Medicated and Unmedicated Parkinson's Disease Patients. CHERYL A. PFEIFFER, RICHARD L. DOTY (Smell and Taste Center, University of Pennsylvania), MATTHEW B. STERN, STEVE M. GOLLOMP, AND HOWARD I HURTIG (Department of Neurology, Graduate Hospital, Philadelphia, PA)*

Recent data suggest that olfactory dysfunction may be among the first signs of idiopathic Parkinson's disease (PD). To determine whether the olfactory deficit is lateralized in PD patients with lateralized motoric features, we tested the olfactory function of each side of the nose of 20 early-stage PD patients not taking antiparkinsonian medications, 20 early-stage PD patients taking antiparkinsonian medications, and 20 matched controls. We assessed, at the time of testing, cognitive function using the Picture Identification Test (PIT) and the degree and laterality of tremor, rigidity, and bradykinesia, as well as gait disturbance, using standard rating scales. Although nearly all of the PD patients evidenced olfactory dysfunction, unilateral deficits were not observed. The same degree of olfactory loss was present in medicated and nonmedicated patients, and the severity of the olfactory deficits was unrelated to such factors as time since disease onset, PIT scores, and the magnitude of motoric disturbance. These findings will be discussed in relation to the hypothesis that some forms of parkinsonism may be caused by airborne agents which damage olfactory function as they gain entry into the central nervous system via the primary olfactory pathways.

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Human Olfactory Biopsy: Comparison of Light and Electron Microscopic Observations from Autopsy Patients. SEOG I. PAIK, MICHAEL N. LEHMAN, DAVID V. SMITH, and ALLEN M. SEIDEN (University of Cincinnati College of Medicine)

The development of a biopsy procedure for human olfactory epithelium (Lovell et al., 1982) has produced a number of investigations focused on ultrastructural abnormalities in patients with olfactory dysfunction (Hasegawa et al., 1986; Jafek & Eller, 1989; Moran et al., 1985). One of the problems with this procedure is the relative difficulty in obtaining olfactory epithelium in each biopsy sample. In order to evaluate potential sampling error in this procedure, we are examining tissue taken with the biopsy technique in relation to the distribution of the olfactory and respiratory mucosa in autopsy patients. We collected specimens of the nasal mucosa from autopsy patients, within 24 hours after death. First, three blind biopsies were taken from the left nasal septum and prepared for transmission electron microscopy (TEM), just as in surgical patients. We then removed a large piece of the nasal septum and superior turbinates, including the cribriform plate, through the anterior cranial fossa. The intact nasal mucosa of the right side was fixed in 10% formalin, divided into five pieces from anterior to posterior, paraffin embedded, and sectioned at 10 *um*. The distributions of olfactory and respiratory epithelia were reconstructed from silver-stained sections of these five pieces. The mucosa of the left side was placed into 1% osmium tetroxide after fixation with 2% glutaraldehyde/0.6% paraformaldehyde. In this preparation, olfactory mucosa is stained a dark black color against a lighter blue-gray respiratory epithelium (Naessen, 1970). Olfactory regions were frequently invaded by patches of respiratory epithelium. Using this *en bloc* osmium stain, we could determine whether the blind biopsy sites were in the appropriate region. Evident in both silver-stained material and in tissue examined with TEM were numerous instances of olfactory epithelium containing only a few or no receptor cells, degenerating supporting cells, and invagination of respiratory epithelium beneath the mucosal surface.

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Olfactory Threshold and Otolaryngolic Examination in Alzheimer's Disease. J.I. FELDMAN, (UCSD Medical Center, San Diego, CA) C. MURPHY, (San Diego State University, San Diego, CA and UCSD Medical Center, San Diego, CA) T.M. DAVIDSON, G. GALINDA, A.A. JALOWAYSKI (UCSD Medical Center, San Diego, CA)

There is considerable evidence to demonstrate that patients with Alzheimer's disease show neuropathological changes characteristic of the disease in olfactory areas of the brain: anterior olfactory nucleus, entorhinal cortex, and piriform cortex. Most recently it has been reported that there are histopathological changes in the olfactory epithelium of patients who died with the disease. Psychophysical studies have indicated that patients with Alzheimer's disease show impairment in a variety of olfactory-mediated tasks. The present study investigated the possible presence of acute nasal disease in 21 patients with Alzheimer's disease and 21 age-matched controls whose olfactory threshold function was clearly defined. All were participants in the UCSD Alzheimer's Disease Research Center (ADRC) and had been diagnosed either as having Probable Alzheimer's Disease or as normal by two different neurologists at the ADRC using the NINCDS-ADRDA diagnostic criteria. Nasal airway resistance was assessed using the anterior rhinomanometric method. Specimens of nasal mucosa were obtained from the inferior turbinate, stained and examined for the presence of goblet cells, eosinophils, neutrophils, basophils, and bacteria. Endoscopic examination of the olfactory epithelium was made noting the condition of the septum and the appearance of the olfactory epithelium. Olfactory thresholds of the Alzheimer's patients were significantly higher than those of controls. Nasal airway resistance did not differ in the two groups, either before or after the decongestant. Numbers of goblet cells, eosinophils, neutrophils, basophils and bacteria were not significantly different in the two groups. Patients and controls did not differ in gross anatomy. However, subjects in either group with abnormal appearing olfactory epithelium had higher thresholds.

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Odor Memory in Alzheimer's Disease. SAMUEL JINICH, ROBBIE RHODES AND CLAIRE MURPHY (San Diego State University and UCSD Medical Center, San Diego, CA)

Recent neuroanatomical evidence for damage to the olfactory areas in Alzheimer's Disease suggests that patients with the disease may show selective olfactory dysfunction. Deficits in odor recognition (Serby et al., 1985; Doty et al., 1986; Koss et al., 1987) and in odor memory (Murphy et al., 1987) have recently been shown to occur early in the degenerative process of Alzheimer's Disease. We sought to determine whether odor memory differed from visual memory in a group of well-characterized patients with Probable Alzheimer's Disease. These patients had undergone and extensive ENT examination to rule out nasal disease. The subjects were 16 persons (mean age = 73.67 SD = 5.03) diagnosed as having Probable Alzheimer's Disease by two independent neurologists at UCSD Medical Center's Alzheimer's Disease Research Center, using the NINCDS-ADRDA criteria. Neuropsychological assessment included the Blessed Dementia Rating Scale (Blessed), the Mini-Mental State Exam (MMS), and the Dementia Rating Scale (DRS). On each of these tests, as the number of errors increases, so does the dementia score. Typical questions on these tests of memory and concentration are "What year is this?" and "What is your birthdate?" Generally, normal elderly controls do not make errors in answering these questions. The Alzheimer's patients in this study missed a mean of 15.38 of 33 questions (SD = 5.75) on the Blessed, and answered correctly a mean of 18.5 of 30 questions (SD = 4.38) on the MMS, and a mean of 102.94 of 144 questions on the DRS (SD = 4.38). Thus they were moderately impaired. Subjects in this study performed a recognition memory task for odors and visual stimuli. ANOVA was conducted to compare odor and symbol Az scores. (Az scores take into account both sensitivity and criterion, since hits, misses, correct rejections and false-alarms are used to compute the score.) Odor memory was significantly impaired relative to memory for faces. The mean Az score for odors was .60 and for faces .85. Chance performance would be measured as .50 on this scale. Clearly the patients with Alzheimer's Disease had difficulty remembering odors. The results will be discussed within the context of recent advances in our understanding of olfactory system involvement in Alzheimer's Disease.

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Missing Ingredients I: Aging and the Discrimination of an Aromatic Flavor.

JOSEPH C. STEVENS, WILLIAM S. CAIN, AND FELICITA REID (John C. Pierce Foundation Laboratory)

This study explored how well young, middle-aged, and elderly subjects could discriminate the presence-absence of the spice marjoram in a carrot soup prepared according to a published recipe. Most young performed the discrimination task at a level above chance, but most middle-aged and elderly subjects failed to do so. A clinical test that entails assessments of threshold and odor identification revealed that olfactory ability also declined with age among these same subjects. Further, the odor thresholds measured in the test correlated significantly with the subjects' ability to discriminate the marjoram flavor. Also, a second discrimination test, like the first except that the nose was blocked to prevent or reduce smelling, confirmed the hypothesis that olfactory ability largely underlay the discrimination; for under this condition the young performed no better than the older subjects. The outcome emphasizes that losses in olfaction usually measured with environmental odors sniffed through the nostrils are sufficiently profound to impair discrimination of everyday foods, even in middle age. Supported by NIH Grant AG04287.

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Missing Ingredients II. Aging and the Discrimination of the Taste of Sodium Chloride. AMY M. RUTHRUFF, JOSEPH C. STEVENS, WILLIAM S. CAIN, AND ANNICK DEMARQUE (John B. Pierce Foundation Laboratory)

Young, middle-aged, and elderly persons attempted to discriminate the presence-absence of salt in a published recipe for tomato soup. All but one young person of 21 tested discriminated above chance level, but more than half of each of the two older groups (20 in each) failed to. Both this discrimination and the absolute threshold for NaCl in water solution turned out to correlate with age from 18 to 89 yrs. These results add to a similar study of the discrimination of an aromatic spice, marjoram [See Abstract "Missing Ingredients I: Aging and the Discrimination of Olfactory Flavor (Marjoram)"] in demonstrating that taste and smell weaknesses documented in recent psychophysical studies can reveal themselves plainly in the perception of everyday food preparations. A secondary study compared thresholds for NaCl in water with NaCl thresholds in the presence of tomato, the principal acidic ingredient of the published recipe. These thresholds, perhaps because of mixture interaction between salty and acidic tastes, were from seven to ten times higher than thresholds in water. Although thresholds for young and old were more nearly alike in the acidic tomato medium than in water, the elderly nevertheless needed over twice as much salt concentration as the young just barely to appreciate its presence. The findings are evaluated in terms of the salt content of several other published recipes calling for tomato and salt, and health and dietary implications are discussed. Supported by NIH Grant AG04287.

Nutritional assessment of elderly persons eating flavor enhanced foods. SCHIFFMAN, SS, FREY, AE, and WARWICK, ZS (Duke University)

The purpose of this study was to determine if flavor enhancement of foods improved the nutritional status of elderly persons. Thirty eight residents of the Methodist Retirement Home in Durham, NC were divided into two groups. Group 1 received food that was unenhanced by flavor for the first 3 weeks and food enhanced by flavor for the second 3-week period. For Group 2, the order was reversed. Eight flavors were utilized throughout the study: roast beef, ham, natural bacon, prime beef, grilled beef, maple, butter, and cheese. Five types of measurements were obtained at the beginning of the study, at the end of three weeks, and at the end of six weeks. These were: biochemical measures (including somatomedin-C/insulin-like growth factor I, transferrin, total T and B lymphocytes, albumin, and creatinine), anthropometric measurements (weight, stature, midarm circumference, and triceps skinfold thickness), taste and smell assessments, functional parameters (handgrip and pinch strength), and measures of well-being (affect balance scale and life-satisfaction index). Measurements of food consumption (plate wastage) were determined for each weekday meal (5 days per week). Flavoring of foods was found to enhance the consumption. However, no significant differences in biochemical measures was obtained. Many elderly residents had intakes that deviated significantly from the RDAs.

Adult-like hedonic responses to odors in 9-month-old infants. HILARY J. SCHMIDT (Monell Chemical Senses Center)

Recent methodological innovations have enabled the demonstration of adult-like odor preferences and aversions in children as young as three years of age thus challenging a long standing view that odor preferences and aversions are not evident until 5 years of age. To test whether odor preferences and aversions may have their origins in infancy, an 'object-odor' exploration task was employed to evaluate 9-month-old infants' reactions to an unpleasant odor, a pleasant odor and a no-odor control.

Infants (9 males and 9 females) were videotaped while exploring in successive 60-90 second trials, three rattle-like toys which were identical except for their odors: one was odorized with butyric acid, one with methyl salicylate, and the other was odorless. The odorants were approximately matched for perceived intensity and trigeminal component, and order of presentation was counterbalanced across infants. Videotapes were then viewed independently by 8 naive adults who were asked to judge the hedonic valence of the odor in each rattle (good, bad, neutral) based on the infants' behaviors.

All 8 adults were significantly better than chance. A micro-analysis of specific behaviors including smiles, frowns, brow-knits, frequency and time spent mouthing, fingering, banging, looking at, and dropping the rattle failed to reveal any single behavior to be systematically associated with an hedonic response to either odor across infants. Different infants apparently convey their hedonic reactions in different manners. Odorized rattles, however, were brought to the mouth and explored in the mouth more frequently than the non-odorized rattle, regardless of quality.

The demonstration of odor preferences and aversions in 9-month-old infants further challenges the behaviorist view that hedonic reactions to odors are acquired via associational learning processes and raises the possibility that some odors are inherently pleasant or unpleasant.

Pica, Chemosensation, and Iron Deficiency, a Pilot Study. WAYNE L. KLEIN (University of Florida) & THOMAS B FAST (University of Florida)

Pica is a form of compulsive eating in which huge quantities of either a single food or a non-nutritive substance are repeatedly consumed for at least one month. Most pica sufferers exhibit iron and/or zinc deficiency. It is frequently argued that pica is an adaptive response serving to increase mineral intake; however, most substances craved are notably lacking in minerals. The alternative suggestion that pica causes the mineral deficiency is not supported by the high frequency in anemic populations of pagophagia or ice pica, a practice unlikely to interfere with iron intake. Additionally, pica quite frequently vanishes following iron therapy. Given the apparent higher incidence of pica in pregnancy and renal disease, two conditions associated with chemosensory changes, the authors hypothesized that pica might be mediated by chemosensory dysfunction secondary to mineral deficiency. Subjects (n=7), all females (mean age 29, range 19-60) exhibited pica for one or more of the following: dry raw corn starch (1 lb/day), ice (10 trays/day), radishes (30/day), clay, and the most serious case, a pregnant attorney who ate ink erasers to appease her craving for gasoline. Based on the Pocket Smell Test, olfaction was normal in all subjects (n=7). Ferritin levels were extremely low in all subjects tested (n=5). Pica vanished in subjects complying with iron therapy (n=3) and ferritin levels increased. At the second testing taste detection and recognition thresholds (3 drop forced choice) were not significantly different in either treated (n=3) or untreated subjects (n=2). There was no evidence for chemosensory dysfunction mediating pica.

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Cephalic Phase Insulin Release in Humans. KAREN TEFF (Monell Chemical Senses Center), RICK MATTES (Monell Chemical Senses Center), Karl ENGELMAN (Hospital of the University of Pennsylvania).

Large variability has been observed in human studies of cephalic phase insulin response (CPIR). Some investigators have postulated that there may be "responders" and "non-responders" in the normal population. To test the reliability of the CPIR, the same food stimulus was administered three times to each subject over 5-days. Normal weight male subjects (n=20) with no history of diabetes were given an aspartame-sweetened mousse composed of gelatin and dairy fat (200 Kcal) after an overnight fast. Four baseline blood samples were taken prior to food administration followed by collections at 2 minute intervals for 16 minutes post-ingestion. Significant increases were found in plasma insulin as relative to mean baseline values (21.4+6.3 uU/ml) at 4 minutes post-ingestion on all three trial days ($p<0.001$, $p<0.02$, $p<0.001$; days 1, 2, 3 respectively). Plasma glucose declined at 4 minutes post-ingestion on the second and third trials only ($p<0.05$, day 2; $p<0.01$, day3). Individual CPIRs were found to be significantly correlated between the three test days. 90% (n=18) of the subjects exhibited a CPIR (value greater than 1 SD of baseline mean) in at least one out of three trials. 50% responded on the first trial and 75% displayed a CPIR by the second trial. Only two subjects did not exhibit a response on any test day. The low percent of non-responders in this population would seem to reflect a normal biological variation in response as has been observed in healthy subjects. Evidence for the existence of a sub-population of non-responders is not substantial. Therefore, we confirm the existence of a reliable cephalic phase insulin response in humans.

Sweet Taste and Energy Intake in Humans. RICHARD MATTES (Monell Chemical Senses Center).

It has recently been argued that sweet taste and non-nutritive sweeteners (especially aspartame) stimulate hunger and food intake. However, inadequate control over sweetener type, energy content and sensory properties leaves this view open to question. Following a one week baseline period, 24 healthy, weight-stable, adults ingested 3 standardized breakfasts (presented in random order) for 5-day blocks. The meal included equicaloric cereals which were either unsweetened or sweetened with sucrose or aspartame. Hunger ratings, food intake and the predominant taste of each ingested item were recorded continuously. Only half of the subjects were informed regarding the type of sweetener presented. Hunger ratings, food intake and the proportion of calories derived from predominantly sweet, sour, salty and bitter items were similar during the three experimental blocks. Individuals who knowingly ingested aspartame tended to have the highest mean daily energy intake. These data fail to support the view that sweetness or aspartame stimulate hunger, energy intake, or influence the predominant taste qualities of foods eaten later in the day. However, the observed tendency to overcompensate for presumed energy savings at one meal, due to non-nutritive sweetener use, suggests an important role for cognitive factors in the regulation of energy intake.

Individual Differences in Perceptions of Selected Gustatory Stimuli and Their Relationships with Food Acceptance. DEIDRE M. BLANK, RICHARD D. MATTES, AND DAVID J. MELA (Monell Chemical Senses Center)

Marked individual differences in perception have been described for a number of chemosensory stimuli. The identification of compounds for which humans demonstrate wide variation in sensory response may aid in characterizing possible physiological and genetic bases underlying variation in sensory perception. Unfortunately, few such marked individual differences have been substantiated for gustatory stimuli, the major exception being the well-documented variation in taste sensitivity to bitter thiourea-type compounds, such as n-propylthiouracil. In addition, there are poorly substantiated reports of wide variation in perceptions of quinine sulfate, sodium benzoate, mannose, and creatine. Taste thresholds and perceived intensity and taste quality were simultaneously determined for all of these stimuli in a group of 16 healthy adults. Gustatory response measures were analyzed in relation to thiourea taster status, food dislikes, and frequency of consumption of selected foods with relevant chemosensory and nutritional attributes.

Tracking Foodstuff Location Within the Mouth in Real Time W. E. LEE III and M. A. CAMPS (College of Engineering, University of South Florida, Tampa, FL 33620)

A computer-based procedure has been developed which allows the tracking of the location of food samples within the mouth in real time. The BASIC program presents an outline of a human upper jaw on the screen which also indicates the location of cheek inner surfaces. Subjects use a mouse to move a cursor on the computer screen to indicate their assessment of where the sample is located in the mouth at any instant in time. Cursor movement is restricted so that movement outside the "mouth" is not allowed. Data is stored in an array of the form: (X-position, Y-position, time). Program output displays the data in the form of a "trail" with each position's order of appearance indicated. This procedure was used to study a variety of food samples, including a beverage, a salted snack, chewing gum, and hard candy. Each of these samples displayed a characteristic temporal "fingerprint". While not absolutely quantitative, the patterns do help discern what activities may be dominating at any point in time such as molar compression and tongue compression. This procedure can be used in conjunction with other sensory textural profiling techniques to understand the possible relationships between the textural attributes and where/when the sample is located in the mouth.

Quantum Chemical Study of Sweet and Anti-Sweet Principles. Molecular Similarities and Chemo-Receptive Activity. DENNIS GERSON AND REICHARD SEFECKA (IBM Corporation)

Similarity Analysis of biologically active molecules is emerging as a method for interpreting subtle differences in molecular properties, such as hydrophobicity, electrostatic potential and polarizability, in structurally similar molecules. The triterpene anti-sweet compounds and their structurally similar sweeteners are examined using the methods of Similarity Analysis (1). The perceived biological activity of these molecules will be related to the electronic molecular properties as determined by Quantum Mechanics methods (molecular orbital theory) and statistical mechanics (Monte Carlo simulation). Additionally, the use of Free Energy Perturbation methods to examine the changes in molecular properties during the "mutation" of an anti-sweet principle into a sweetener will be presented and discussed.

(1) Compounds include Jujubogenin, Gymnemagenin, Glycyrrhizin and Steviol.

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An Experimental and Computational study of the Relationship between the Volumes and Propoerties of Sweet Molecules. I. BARTOLO, M. G.B. DREW AND G.G. BIRCH (University of Reading, Whiteknights, PO Box 226, Reading RG6 2AP (UK)

Molecular volumes of aldopyranoses (in the $^4C_1$ conformation) and other sapid molecules were computed using the Gepo187 program. This program generated surface areas by probing with a solvent (water) molecule and hence Van der Waals Volune (Vv), Molecular Volume (Vm) and Accessible Molecular Volume (Va) were calculated and shown to follow the pattern Vv<Vm<<Va. Apparent Molecular Volumes of sugars (Vam) have been determined experimentally by a variety of techniques including precision densitometry. The experimental/calculated ration (Vam/Vm) is constant at 1.14 $\pm$ 0.02 for different sugars. The value of Va was consistently larger than that of Vam. The calculated/experimental ratio (Va/Vam) is constant at 2.84 $\pm$ 0.07 for different sugars. No significant differences (<0.5%) were observed in computed volumes of anomers or rotamers of the sugars with different 3C-4C-4O-4H torsion angles. The same trends occurred for values of both Vv and Va. Thus for sugars, the calculated Vm volume is a good indication of the experimental apparent molecular volume. While the Vam/Vm ratio for sugars is constant within a narrow band, the same is not true of the amino acids where the raio ranges form 0.916 to 1.17. The different ratios can be related to variations in the hydrophobic and hydrophilic nature of the amino acids and can be used to give an indication of the likely taste of the acid.

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Solute-Water Interactions as Studied by Nuclear Magnetic Resonance Spectrometry: A Contribution to Taste Investigations.

JOHN M GRIGOR and GORDON G BIRCH
Department of Food Science and Technology, University of Reading, Whiteknights, PO Box 226, Reading RG6 2AP (UK).

Recent advances in nuclear magnetic resonance spectroscopy (NMR) are reviewed in relation to molecular recognition. 17 L-amino acids are examined by NMR techniques to study their effects on water structure. ^1H spin-spin (T2), and spin-lattice (T1) relaxation rates are measured on a low resolution pulsed NMR spectrometer for several concentrations of each amino acid (some amino acids however have limited solubilities). T2 and T1 both decrease with concentration. However the rate of decrease is dependent on structure of the particular amino acid. T2 is also dependent upon the proton exchange rates which occur between water molecules and solute molecules. The exchange of protons is induced by hydrogen bonds providing channels for exhange to occur. Receptor events for sweet and bitter tastes are mediated by hydrogen bonding. The question is therefore whether the triggering of the receptor is responsive to the changes in water structure as initiated by the tastant. As pH variations induce motional and conformational changes in amino acids, the T2 of water, as disturbed by the solute is examined over the pH range 1 to 12.5. The pH of the 17 amino acids, made up at a concentration of 0.6% w/w and with no acid or base added, range from 3.2 to 10.1. The characteristic minimum T2 of water near pH 7 is altered and displaced differently by each amino acid. This minimum T2 value appears to be positively correlated with apparent specific volume. NMR studies therefore aid investigation of the effect of sapid solute on water structure.

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Open Label Trial of Phosphatidylcholine for Olfactory and Gustatory Problems. HIRSCH, ALAN R. (Smell & Taste Treatment and Research Foundation)

Ten patients underwent trial of 9 gm per day for a three month period of oral PhosChol. PhosChol is a highly purified form of phosphatidylcholine. Age range of patients was from 35-80 with an average age of 55. All patients had complaints of impaired ability to smell and taste. Three of the patients also had complaints of parageusia. All patients underwent comprehensive blood testing including B12 level, red blood cell folate level, FTA, ESR, CBC, platelets, glucose, liver function tests, electrolytes, 24-hour urinary MHPG level, total eosinophilic count, IgE level, PT, PTT and platelets. All patients were studied with brain mapping, FFT analysis, P300 analysis, brainstem auditory evoked responses, EEG, and visual evoked responses. All patients had smell and taste tests including unilateral carbinol testing, taste threshold and suprathresholds to sodium chloride, hydrochloric acid, sucrose, urea, and PTC; taste quadrant testing; unilateral phenol testing; Upsit; unilateral PD lactone testing; unilateral Connecticut Home Olfactory Testing; unilateral cineole testing; unilateral electric gustometry testing; unilateral thiophane testing; unilateral pyridine testing, unilateral phenone testing; smell suprathreshold intensity testing; smell suprathreshold adaptation testing; smell suprathreshold detection testing; smell suprathreshold recognition testing; smell suprathreshold identification testing. All patients also underwent psychological testing including MMPI testing, MCMI testing, and Beck Depression Inventory.
Results of Study: Forty percent (4 of 10) had subjective improvement of sense of smell with use of phosphatidylcholine. Predictive indicators of positive response will be presented.

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Electrophysiological Indicators of Laterality in the Human Olfactory System James D. Drash, U.S. Environmental Protection Agency

Anatomically, the olfactory system is unique in a variety of ways. One important aspect of this uniqueness is that its primary sensory projections are ipsilateral rather than contralateral as in the somatosensory system. The use of an evoked potential paradigm can reveal information about the unilateral processing of olfactory stimulation. To this end olfactory stimulation was delivered to eight human subjects who were trained to hold their breath while keeping the mouth open during stimulation. At other times the subjects breathed gently through the mouth. The contralateral, unstimulated naris was plugged. Thus the stimulus entered the left naris and exited the oral cavity. The stimuli, 16000 ppm spectrophotometric grade toluene, were delivered intranasally for 500 msec from an olfactometer. Fifty trials were delivered on a pseudorandom basis of 6 to 10 sec. EEG electrodes were placed at F7, Fz, and F8 and referenced to linked mastoid processes. Data reduction consisted of subtracting the evoked potential obtained at Fz from F7 and F8. The area under the curve of the resultant waveforms, F7-Fz and F8-Fz, was calculated. A one-tailed t-test ($t=6.593$, $df=7$, $p<0.0005$) indicated that there was significantly more activity in the hemisphere ipsilateral to the stimulated naris. These data provide electrophysiological confirmation of the olfactory anatomy and evidence that the processing of olfactory information presented to one naris is ipsilateral. In addition, had the stimulus been a strong trigeminal stimulus one would expect to record more electrophysiological activity from the hemisphere contralateral to the stimulation. The bimodal peak obtained may reflect the olfactory anatomy mediating olfactory processing. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

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Chemosensosry Evoked Potentials in Patients with Olfactory Disturbances. TH. HUMMEL, H. PIETSCH, TH. MOKRUSCH, and G. KOBAL, Dept. of Pharmacology and Toxicology, University of Erlangen-Nürnberg, Universitätsstrasse 22, D-8520 Erlangen, West-Germany

One of the major problems in olfactometry has always been how to obtain data which exceed subjective reports - comparable to near field and far field potentials routinely recorded in audiology. In our laboratory a method was developed by the aid of which it became possible to obtain olfactory evoked potentials (OEP). But only by employing multichannel recordings and magneto-encephalographic methods we were finally able to distinguish between the specific patterns of those responses elicited by stimulation of the trigeminal nerve and those evoked by stimulation of the olfactory nerve. OEPs tend to be distributed at parietal-central sites of the skull, whereas trigeminal responses (chemosomatosensory evoked potentials CSSEP) can be found at central-frontal positions. The ability to localize the site of stimulation, which differs according to olfactory or trigeminal stimulation (Kobal et al., "Is there Directional Smelling?", Experientia 45, 130-2, 1989), can be helpful in diagnosing anosmia. Thirty anosmic patients were investigated in this study. EEG was recorded from 8 positions of the international 10/20 system. Whereas CSSEPs were always recorded, olfactory stimuli yielded no responses. Several cases of hyp- and anosmic patients will be demonstrated, as well as the course of transient anosmias caused by influenza. In addition, patients suffering from Alzheimer's and Parkinson's disease were also investigated.

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First recordings of the cognitive component P300 using olfactory stimuli. G. KOBAL and M. DURAND-LAGARDE, Dept. of Pharmacology and Toxicology, University of Erlangen-Nürnberg, Universitäts-strasse 22, D-8520 Erlangen, West-Germany

The cognitive component P300 in event-related potentials (ERP) is usually recorded in humans responding to series of low probability stimuli during a discrimination task. The aim of the present study was to demonstrate that this type of endogenous cortical response can also be recorded in olfactory discrimination tasks, since we were abble to solve the problem of short stimulus intervals. 16 volunteers (8 male and 8 female) participated in the experiments, which consisted of 4 different, randomized sessions. A mixture of vanillin and H_2S was used as standard stimulus ($p=0.85$, 200 msec duration). Target stimuli ($p=0.15$) were either H_2S or vanillin. Stimulus intervals could be reduced to 8 sec. This still allowed the subjects to perceive stimulus onsets. Both types of targets were used for stimulating the right and the left nostril. In addition to the discrimation task (counting the rare stimuli) subjects had to indicate the pleasantness/unpleasantness and the intensity of the stimuli. EEG was recorded from 7 positions (10/20 classification) referenced to linked earlobes. Under these conditions P300 components could be recorded in responses to the rare stimuli in all subjects. Amplitudes tended to be larger when the right nostril was stimulated with H_2S. Latency shifts of the early components of the olfactory evoked potential (OEP) correlating with hedonic estimates could be reproduced (Kobal et al., 1989, Chemical Senses, 14, 5). In contrast, the cognitive component P300 did not reveal a significant correlation with hedonic estimates. Recording of P300s promises to be a useful application in testing olfactory discrimination capability, e.g. in Parkinson patients.

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Odor and Cognitive Modulation of the Contingent Negative Variation. Tyler S. Lorig & Melissa Roberts (Washington & Lee University)

EEG techniques may be useful in understand odor effects but care must be exercised in the interpretation of these findings since non-odor related effects may also alter the EEG. In the present investigation, 18 students participated in a study designed to differentiate cognitive/ expectancy effects from direct physiological odor effects using a CNV paradigm. EEG activity was recorded during each trial. A trial consisted of a 4 second cued odor administration followed by a ready signal (tone) and an imperative signal (visual) to which subjects responded. The cue during the odor administration phase told subjects that they were smelling either odor A, B, C, (primary odors) or no odor. These odors were jasmine, galbanum and lavender, respectively. In addition to these odors, subjects were also cued that lower concentrations of these odors were being administered. In this case however, all three odors were an identical mixture of the primary odors (mixture). Thus, subjects were cued to expect different odors but identical odors were presented. Post-experiment questioning revealed that all subjects believed that odors cued as low concentrations differed from one another. Each odor condition was presented 10 times and the CNVs averaged. Amplitude of the early CNV was extracted from the averages and compared using analysis of variance. Results indicated a trend toward differences among primary odors ($p=.076$) and a significant difference among responses to the odor mixture ($p=.023$). Topographical analysis of these odor effects indicated similar topographies for each primary odor and its "supposed" lower concentration while large differences were noted between individual odors. These data indicate that odor modulated CNV effects can be due to cognitive or expectancy effects rather than any direct odor effect such as CNS arousal.(IFF provided grant support for this research.)

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Human Infant Olfactory Processing - the Brain Electrical Activity Mapping (BEAM) Technique. MARTIN KENDAL-REED (University of Warwick), STEVE VAN TOLLER (University of Warwick)

Relatively little is known about how human babies perceive odours, despite over a hundred years' of research. The other sensory modalities have received considerable attention from psychologists, but this has not been the case, until recently, with the sense of smell. This poster will outline some of the early work in infant olfactory psychology before attempting to define the state of knowledge at present. Recent work using a novel technique will then be described. The BEAM method (Brain Electrical Activity Mapping) is a development of traditional EEG psychophysiological techniques. It shows great promise in helping to understand the complex cortical processes underlying odour perception in human infants. Results so far suggest that twelve-week old subjects are able to differentiate food odours in terms of cortical activity. Current research using a sensory-impoverished, low ambient odour testing environment will be described.

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Thresholds for Odor and Pungency J. ENRIQUE COMETTO-MUÑIZ* and WILLIAM S. CAIN (John B. Pierce Foundation Laboratory and Yale University, New Haven, CT 06519).

The study measured detection thresholds repeatedly for 11 odorous chemicals in normals and anosmic persons. Eight of the chemicals comprised the first eight members of the series of n-aliphatic alcohols, and the three others comprised phenylethyl alcohol, pyridine, and menthol. Questions of interest concerned a) whether all 11 materials would prove detectable by the anosmics via pungency, and b) whether the difference between the thresholds of normals and anosmics would vary systematically across materials, particularly in the aliphatic series. The anosmics could detect all but phenylethyl alcohol reliably. In the aliphatic series, both odor and pungency thresholds declined with chain length in a way that implied dependence of both in part on phase distribution in the mucosa. The thresholds for odor, however, declined more rapidly than those for pungency. Accordingly, the ratio of the thresholds of anosmics to those of normals increased through the series, ranging from a low of 12 for methanol to a high of 10,000 for 1-octanol. Because the exponents of the psychophysical functions for the aliphatic alcohols are relatively low and because they also decline with chain length, the gap between the thresholds of the normals and those of the anosmics would translate into a smaller and more uniform change in perceived intensity. Indeed, the calculated change, based upon Laffort's tabular values of exponents, showed no tendency to vary systematically with chain length and raised the intriguing possibility that the threshold of pungency occurs at a criterion level of perceived magnitude, irrespective of the stimulus. Such an outcome seems to harmonize various threshold and suprathreshold responses to organic vapors.

* Member of the Carrera del Investigador Científico, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), República Argentina.

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Cross-Sensitization and Desensitization Between Capsaicin and Piperine: Evidence of Partial Independence of Sensory Mechanisms. BARRY G. GREEN (Monell Chemical Senses Center)

It is well known that repeated topical applications of capsaicin can produce a temporary desensitization to subsequent applications of itself or other chemical irritants. We recently reported that on the tongue, application of capsaicin stimuli at the rate of about 1/min can also produce the opposite effect of sensitization. The objectives of the present study were (1) to determine if piperine, the pungent ingredient in black pepper, would produce similar effects, and (2) if capsaicin and piperine could be shown to cross-sensitize and cross-desensitize. In the first experiment, 10 capsaicin or 10 piperine stimuli (in concentrations that produced approximately equal levels of perceived intensity) were presented to the tongue tip on filter paper disks at the rate of 1/min. After a 15 min hiatus, a final stimulus was presented that was either the same or different than the preceding "conditioning" stimuli. It was found that, like capsaicin, piperine was capable of producing both sensitization and desensitization to itself; however, sensitization was more rapid and stronger for capsaicin, and capsaicin produced a more complete desensitization both to itself and to piperine. In the second experiment, cross-sensitization was measured by applying a series of low-intensity stimuli (also via filter paper disks) of one compound followed immediately by a series of equally intense stimuli of the other compound. The results indicated that cross-sensitization occurred in only one direction: the perceived intensity of irritation produced by capsaicin was significantly higher when stimulation followed five exposures to piperine. Together the results from the two experiments imply either that capsaicin and piperine stimulate some but not all of the same nociceptors, or that they stimulate the same nociceptors but via somewhat different mechanisms. Experiments are continuing to evaluate the possible effects of stimulus intensity on the observed asymmetries.

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Oral Capsaicin Desensitization and its Effects on Taste. TRACY KARRER (Yale University), LINDA BARTOSHUK (Yale School of Medicine)

It has been previously reported that a 10 min application of 10,000 ppm capsaicin (CAP) to the human tongue resulted in desensitization (DESEN) to subsequent lower concentration CAP applications, whereas there were no changes in taste recognition thresholds (Szolcsanyi & Jancso-Gabor, 1973). This abstract reports that a 15 min application of 100 ppm CAP also results in DESEN, and that this DESEN produces decrements in suprathreshold taste. Tests of the perceived taste and tactile intensity of three concentrations of NaCl (.1, .32, 1 M), sucrose (.1, .32, 1 M), citric acid (.0032, .01, .032 M), QHCl (.0001, .00032, .001 M), 6-n-propyl-2-thiouracil (PROP) (.00032, .001, .0032 M), and the perceived burn intensity of four concentrations of CAP (1, 10, 100, 1000 ppm) were performed prior to DESEN, as well as 1 hr, 1 day, and 2 days subsequent to DESEN. An ANOVA showed that all post-DESEN tests of CAP burn intensity yielded lower ratings than did the pretests. All CAP concentrations showed a concentration-dependent decrement in intensity at the 1 hr test, followed by partial concentration-dependent recovery at the 1 day test. The 2 day test showed virtually no additional recovery. Overall, the 1 hr and 1 day, but not the 2 day, taste ratings were lower than the pretest taste ratings. Although the taste x DESEN interaction was not significant, comparisons of means revealed that the two bitters, particularly PROP, showed greater differential sensitivity to test day. In fact, the recovery pattern for PROP taste showed a decrement and recovery similar to that reported for CAP. Overall, for the four subjects who reported a tactile component for some tastes, all post-DESEN tests of the tactile components were lower than the pretests.

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The Effects of Ethanol and pH on Sourness, Bitterness and Astringency intensity and temporal perception. U. FISCHER and A.C. NOBLE (University of CA, DAVIS).

Eighteen wines, differing in ethanol (8, 11 and 14% v/v), pH (2,9, 3.2, and 3.9) and (+)catechin (100 and 1400 mg/L) were prepared using a dealcoholized wine concentrate. In a completely randomized design, bitterness and sourness intensity were rated in duplicate by 21 subjects. Bitterness intensity was increased by the largest magnitude by the addition of ethanol. Raising ethanol from 8 to 11% or 11 to 14% produced a larger increase in bitterness than addition of 1400 mg of catechin. The pH increase from 2.9 to 3.2 also enhanced bitterness, though a further rise diminished perceived bitterness. Sourness decreased with increasing pH, but was unaffected by addition of ethanol or catechin. In a second experiment, 18 wines varying in ethanol (1, 8, and 14%), pH (3.0 and 3.6), and added tastant (none, catechin or tannic acid [1500 mg/L]) were evaluated by time-intensity (T-I) methodology by 24 subjects. Time to maximum intensity (MAX), MAX and duration of bitterness increased with ethanol concentration or addition of catechin and tannic acid. Astringency, which was highest in tannic acid solutions persisted far longer than bitterness. Astringency MAX was slightly decreased by addition of ethanol. In a third experiment using 11 subjects, bitterness of the 1 and 14% ethanol solutions from Expt. 2, were evaluated by T-I, while saliva was collected from the parotid gland. The largest increase in salivary flow was produced by lowering pH. Tannic acid addition elicited a significant increase in flow, while flow increased only slightly with catechin, which was primarily bitter; an increase in ethanol had no affect of salivary flow.

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Qualitative and Quantitative Perceptual Attributes of Astringent Substances. HARRY T. LAWLESS, CHRISTOPHER B. LEE and RICHARD A. TUCCIARONE, (Department of Food Science, Cornell University)

The category of flavor experiences referred to as "astringent" may involve complex sensations of both a chemical and tactile origin. In spite of their importance in food flavors, the nature of astringent sensations remains poorly characterized from a psychophysical perspective. However, there is some agreement as to the use of several substances (e.g. tannic acid, alum, tartaric acid) as good examples of astringent materials in applied flavor work.

Qualitative group discussions were held during which solutions of tannic acid, tartaric acid and alum were tasted, and language appropriate to describe the sensations arising from these compounds was determined on the basis of group consensus, inter-group consistency as well as by reference to previous literature. A composite ballot consisting of six rating scales was derived and used in further psychophysical ratings. Attributes were roughness (of the mouth), dryness, puckeryness (a tightening or drawing sensation), overall astringency, sourness and bitterness. Two concentrations each of tannic acid (3.0 and .75 g/ml), tartaric acid 1.0 of 0.5 g/l) and alum (1.0 and 0.5 g/l) were chosen based on informal (benchtop) similarity in overall sensory impact. Each solution was rated on the six attributes for six minutes following rinsing and expectoration. All ratings showed a roughly exponential decay over time. The intensity ratings for each attribute were found to depend upon concentration and the particular astringent substance. Different substances exhibited different profiles across attributes, especially with respect to bitter and sour side tastes.

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Taste Bud Quantification in Human Vallate and Foliate Papillae. INGLIS MILLER, JR., RUOYU XIAO, and ROBIN KRIMM (Dept. Neurobiology & Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC)

Large variations in fungiform taste bud density occur among different human tongues. This report contains numbers of taste buds in human vallate and foliate papillae from cadaver tongues in which taste bud densities have been reported for the tongue tips and midregions. By choosing different individuals with high and low taste bud densities in the fungiform papillae, we wanted to see whether the relative numbers of taste buds in the vallate and foliate papillae vary with the fungiform papillae or independently of them. Tissue samples were removed from 8 human cadaver tongues as follows: 2 samples with one vallate papilla each, and 1 sample containing a portion of one foliate papilla. An entire foliate papilla was too large to process. The samples were serially sectioned in paraffin, stained in H & E and examined by light microscopy. Taste buds were followed through serial sections and counted only in sections which contained a taste pore. The number of taste buds /vallate papilla ranged from 0 - 481 with a mean of 171.1 ± 168 tb/pap. Samples containing a portion of one foliate papilla had from 1- 5 fissures with taste buds. These contained from 113 to 783 taste buds, which averaged 337.8 ± 210 taste buds per/20 mm^2 of foliate surface. Taste bud distributions in vallate and foliate papillae were uncorrelated with the densities of fungiform taste buds of individual subjects. These data provide profiles of taste bud distribution in 4 regions of the human tongue. We thank tissue donors for the opportunity to study this material. Supported by NIH Grant NS 20101.

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Immuno-Electron Microscopy of Glutamate-Containing Nerve Fibers in the Taste Bud of Mudpuppy (Necturus maculosus) KUO-SHYAN LU and STEPHEN D. ROPER, Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523 and the Rocky Mountain Taste and Smell Center, University of Colorado, Medical Center, Denver, CO 80262

The distribution of glutamate(Glu)-immunoreactive(IR) nerve fibers in the mudpuppy taste bud at the light microscopic level has been reported by our laboratory recently. The present investigation attempts to locate Glu-IR nerve fibers at the electron microscopic level. Lingual epithelium of mudpuppy was dissected out in an aldehyde fixative immediately after decapitation. Thick sections (100 μm) were cut and processed for pre-embedding immuno-cytochemical localization of Glu immunoreactivity. They were treated with monoclonal Glu-KLH conjugated antibody (dilution 1:4,000), biotinylated secondary antibody and avidin-biotin horseradish peroxidase complex. Both extra- and intragemal Glu-IR nerve fibers in the taste bud were observed in thick sections. Ultrastructural observations revealed that numerous profiles of unmyelinated nerve fibers were located at the base and between the taste cells of the taste bud. Nerve profiles at the base of the taste bud were larger in size (1 μm in diameter or larger) and the majority of them (ca. 80%) contained flocculent Glu-IR reaction product. Smaller nerve profiles were observed between the taste cells and about 60% of them were Glu-IR. Small clear vesicles (50 nm in diameter) and occasional few large dense cored-vesicles (150 nm in diameter) were frequently observed in the Glu-IR nerve profiles. Glu-IR synapses have not yet been observed. From the distribution and pattern of the Glu-IR nerve fibers obtained in the thick and thin sections, we concluded that the taste bud of the mudpuppy is innervated by the glutamate-containing unmyelinated nerve fibers. Immuno-gold staining and possible localization of Glu-IR material within synapses of the mudpuppy taste bud is in progress.

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Pre- and postnatal development of fungiform papillae and their innervation in hamster. MARK C. WHITEHEAD AND DIANE L. KACHELE (Ohio State University).

The number and topography of fungiform papillae and taste pores were determined in hamsters aged embryonic day 10 (E10) through adulthood by staining the tongue surface with toluidine blue. Fluorescent dye-tracing experiments (Lasiter, personal communication) evaluated separately the distribution of the chorda tympani (CT) or lingual trigeminal (LV) nerve at each age. The tongue originates as lingual swellings at E10. By E11, a small well-formed tongue, devoid of papillae, has developed with labeled LV and CT fibers present in a large nerve bundle deep in its base. At E12, dome-shaped precursors of fungiform papillae first appear flanking the intermolar eminence. Labeled LV fibers course dorsally and terminate selectively at the epithelium of each fungiform dome. Chorda tympani axons, although reaching the tongue tip, remain deeper as small bundles of labeled fibers that terminate below the epithelium and the fungiform domes. The number of fungiform papillae increases eight-fold from E13 to near term at E15 by sequential addition of more rostral and lateral papillae. During this developmental period, the papillae become more heavily innervated by LV fibers, many of which now terminate intraepithelially in the lateral portion of the papillae. Sparse collections of CT fibers reach the epithelium of the more rostral fungiform papillae by E13 and enter the basal portion of some papillae at E14 and E15. By the end of the first postnatal week, fungiform papillae are adultlike in number and approximately 80% contain buds. Interestingly, development of fungiform papillae occurs in a caudal to rostral manner; a gradient that appears closely paralled by LV innervation. CT innervation of the papillae occurs later and is far less dense than trigeminal innervation. These results demonstrate that fungiform papillae development occurs in a sequentially ordered manner in the pre- and early postnatal hamster. The time-course for innervation and morphogenesis of fungiform papillae suggests that papillae development may be influenced by, or influence, innervation by the lingual trigeminal nerve. It remains to be determined whether taste bud formation may facilitate, or be facilitated by chorda tympani innervation.

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Posterior Diencephalic Connections of the Gustatory System in the Catfish. C.F. LAMB and J. CAPRIO. Dept. Zool. & Physiol., Louisiana State University. Baton Rouge, LA 70803-1725.

To investigate the relationships between the medullary and diencephalic nuclei of the gustatory system of catfish, we identified specific connections with restricted injections of HRP and recorded neural activity of cells within each of the nuclei of the posterior diencephalon. Channel catfish (12-21 cm, 35-105 g) were used for both anatomical and electrophysiological experiments. HRP was iontophoretically injected into the vagal (VL) and facial (FL) lobes, secondary gustatory nucleus (nGS), or inferior lobe (LI), and reacted 2-5 days later using the Hanker-Yates or TMB protocol. Neural activity was recorded by glass microelectrodes filled with pontamine sky blue (2% in 0.5 M Na-acetate, 1-4 Mohm). While recording, portions of the oropharyngeal cavity and extra-oral body surface were stimulated mechanically and chemically (solutions of liver extract, amino acids, or nucleotides). Recording locations were iontophoretically marked and later reconstructed histologically. Our HRP results confirmed earlier reports, and identified nGS efferents terminating in the nucleus centralis of the inferior lobe (nCE) as the rest of the tertiary gustatory tract passed to the caudal and rostrolateral nucleus diffusus (nDLI). Cells in nCE and the caudal portion of nucleus lobo-bulbaris (nLB) responded to tactile stimulation of the bilateral extra-oral surface, but not the oropharyngeal cavity. Cells in the rostro-lateral nLB had predominantly ipsilateral receptive fields (RFs), with many being restricted to the ipsilateral mouth, head, or oropharyngeal cavity. Two recording sites in this region contained units that were stimulated by ipsilateral oropharyngeal stimulation and were inhibited by extra-oral stimulation. Cells in nucleus lateralis thalami (nLT) and the rostrolateral nDLI were responsive to ipsilateral oral and extra-oral stimulation. These results provide functional support for anatomical evidence that these diencephalic nuclei form differential feedback loops with the primary (VL & FL) and secondary (nGS) gustatory centers in the teleost brain and that the nGS might have two functionally distinct projections.

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Differences in distribution of sialated glycoconjugates in secretory cells of the salamander olfactory mucosa. JAMES D. FOSTER, MARILYN L. GETCHELL* & THOMAS V. GETCHELL, Depts. of Physiology & Biophysics and *ENT Surgery, University of Kentucky College of Medicine, Lexington, KY 40536.

Fluorescein-labeled lectins were used to characterize the distribution of sialic acid, galactose (Gal) and N-acetylgalactosamine (GalNAc) residues in glycosylated secretory products of sustentacular cells (SC) and acinar cells of Bowman's glands (BG) of the olfactory mucosa of tiger salamanders. FITC-Peanut lectin (PNA) which binds preferentially to terminal Gal beta 1,3 GalNAc residues of oligosaccharides labeled the apical region of acinar cells with moderate intensity but only weakly stained the apical region of SC. Neuraminidase treatment dramatically increased the staining intensity in the apical region of SC while staining in BG was unaltered. These results indicate that secretory products of SC are sialated and that at least some of the sialic acid residues found in SC are attached to and cap subterminal Gal beta 1,3 GalNAc residues. This confirms and extends previous results from this laboratory using histochemical techniques to localize mucopolysaccharides. Results obtained using Soybean (SBA), Dolichos biflorus (DBA), Griffonia simplicifonia I (GSI) & Maclura pomifera (MPA) lectins suggests that terminal Gal and GalNAc are present in glycoconjugates of both BG and SC. Two lectins, SBA and Bauhinia purpurea (BPA) labeled superficial BG more intensely than the deep olfactory glands indicating differences in cellular processing of glycoconjugates by these two populations of glands. Pre-incubation with the appropriate sugar resulted in suppression of staining by the lectins used in this study. These results demonstrate that glycoconjugates secreted by SC and BG in the olfactory mucosa are different in their saccharide compositions, and these differences may correspond to unique functional roles of these secretions in perireceptor events.

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Pokeweed Agglutinin Labels a Subpopulation of Olfactory Receptor Neurons in Rainbow Trout. DAVID R. RIDDLE and BRUCE OAKLEY (Neuroscience Prgm. and Dept. Biology, University of Michigan).

We have investigated the binding of several lectins as part of a study of the organization of the olfactory system of rainbow trout, *Salmo gairdneri*. We report here that pokeweed agglutinin (PWA) binds to a subset of olfactory receptor neurons scattered throughout the mucosa and to axons that are widely distributed in the olfactory nerve (ON) over most of its 1cm length. At the level of the olfactory bulb (OB), however, PWA stains axon fascicles and terminals that are spatially segregated. Paraffin sections of the olfactory mucosae, olfactory nerves, and brains of ten hatchery raised adult trout were stained using biotinylated PWA and an avidin-biotin-peroxidase complex. On each lamella of the olfactory rosette, PWA intensely stained the entire extent of some olfactory receptor neurons, including the basally directed axon, the apical dendrite, dendritic knob and cilia. The intensely stained cells were intermingled with receptor cells in which only the dendrite was stained. Darkly stained axons, intermixed with lightly stained or unstained axons, were evident in the axonal bundles in the connective tissue core of each lamella and in the olfactory nerve proper. PWA also stained axons in the olfactory bulb. The glomerular layer (GL) was densely stained in the dorsolateral OB and in restricted regions of the posterior OB. The anteriomedial OB was lightly stained, whereas the GL of the lateral and ventrolateral OB was unstained. We labeled the primary olfactory afferents in some fish with HRP, and stained the fibers immunocytochemically in others. We found that the PWA negative regions of the GL were well innervated, supporting the conclusion that PWA binds to a subset of olfactory fibers in the OB. The discrete pattern of differential staining was bilaterally symmetrical and consistent among fish. This remarkable organization apparently arises as the ON enters the OB, since PWA positive axons are widely scattered in the ON, but segregate into intensely stained fascicles as they near the OB and innervate specific regions of the GL. These results demonstrate a geometrically ordered non-topographic primary olfactory projection which offers an opportunity to study how diffusely organized axons aggregate and terminate in selective regions of the olfactory bulb.

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Transmembrane Currents in Frog Olfactory Cilia. STEVEN J. KLEENE and ROBERT C. GESTELAND (Department of Anatomy & Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267).

We have measured transmembrane currents in intact single cilia from olfactory receptor neurons of *Rana pipiens*. One cilium on an isolated receptor neuron was sucked into a patch pipette (tip diameter 0.5 μm), and a high-resistance seal was formed near the base of the cilium 48% of the time (n=661). Input resistance averaged 2.0 GΩ. Measurements were done under voltage-clamp. At rest, with the cell attached by the cilium in the pipette, there was a current averaging 10 pA into the pipette. Fourteen of 91 cells tested responded to an odorant mixture. The response was detected as a transient increase in current into the pipette at negative pipette potential. The peak current increased with increasing negative pipette potential and with stimulus duration. Response latency was variable, and the induced current typically lasted 10 sec. No induced current was seen with the pipette potential at 0; results at positive potentials have been inconclusive. With a 1-sec stimulus and a pipette potential of -80 mV, the average peak response was 42 pA. Some cells inactivated after a single response, while other showed no decrementing with repeated stimulation for at least 15 min. In 2 cells, a single action potential was seen at the start of a response. When a pipette with cell attached was raised into the air and then reimmersed, the cell was pulled off, leaving a single cilium sealed inside the pipette. A resting current into the pipette, averaging 13 pA, was still observed. Of 158 excised cilia, 79 showed increased conductance after application of 1 mM cAMP to the bath. This suggests that the cytoplasmic face of the ciliary membrane was exposed to the bath. The cAMP-induced current was much greater at negative than positive pipette potentials and did not discriminate between Na and K ions. Of the 79 excised cilia that responded to cAMP, the input resistance before stimulation averaged only 0.51 GΩ. It may be that calcium in the bath solution increased the ciliary membrane conductance. If not, it remains to be explained how the soma maintains a high input resistance with several high-conductance cilia attached.

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Patch Recording Implications for Olfactory Transduction. ROBERT C. GESTELAND, STEVEN J. KLEENE and RAYMUND PUN (University of Cincinnati College of Medicine, Cincinnati, OH 45267).

Measurements of currents in patch-clamped cilia and of membrane excitability in whole-cell patch recordings of frog olfactory receptor neurons raise doubts about the adequacy of conventional hypotheses to account for transduction processes. Resting-state conductance of the ciliary membrane is high. Opening of channels by odors should cause only small changes in transmembrane current. Mucus which bathes the cilia has higher potassium concentrations than that which probably bathes receptor neuron somata. The ciliary membrane should depolarize the soma and axon and inactivate them. This effect is likely to be strong because the surface of the cilia constitute about 25% of the cell surface area. Neighboring cilia should present low-resistance shunts to each other, reducing electrotonic spread of receptor currents to the soma and axon. However, measurements of input resistance at the soma do not show the high conductances of the cilia. Excitability of the dendrite-soma membrane is low at normal resting potential. The membrane potential for 50% inactivation is tens of millivolts more negative than in most other cells of the nervous system, and the slope of the inactivation function is unusually steep. To obtain action potentials in the soma in response to odors, it was necessary to remove inactivation by passing hyperpolarizing current through the recording electrode.

An unconventional model of the frog olfactory receptor is consistent with the experimental observations. The cilia appear to the cell soma to be infinite-impedance current sources which are activated by odors. When activated they inject current into the soma. The inactivated membrane of the dendrite and soma causes odor-evoked currents from the cilia to flow preferentially out through the axon and hillock. This is an efficient process for translating receptor currents into increases in action potential rate. Since the cilia are current sources, their currents summate and current from one cilium is not dissipated in its neighbors. This model predicts a receptor with high sensitivity and high efficiency. It probably has a slow temporal response. It suggests that the cilium is a biochemical transducer rather than an electrical cable operating via the principles of Nernst, Kirchhoff and Maxwell.

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An Investigation of Taste-Smell Interactions Across Four Tastants and Six Odorants. G. SHAFFER and R. A. FRANK (University of Cincinnati)

Previous studies have shown that taste-smell interactions are tastant and odorant dependent (Frank & Byram, Chemical Senses 13:445-455). The scope of those studies was limited to the influence of two odorants on two tastants. In the present study sucrose, sodium chloride, citric acid, and quinine sulphate were combined factorially with almond, chocolate, lemon, peanut, strawberry, and wintergreen flavoring to assess taste-smell interactions within all for taste qualities. Pilot testing indicated that all six flavors had no perceivable taste at the concentrations used in the study. Each of the 24 combinations plus the four tastants, six odorants, and distilled water were judged twice by 16 subjects in two sessions. All judgements were made on a 21 point category scale on which 0 indicated no perceivable taste and 20 indicated a very strong taste. Prior to the experiment, subjects sampled the four tastants and distilled water so that they would have a context for their ratings. During the experiment subjects swallowed 2.5 ml samples of each stimulus and rated the total intensity of the taste, then the intensity of the components (sweet, salty, sour, bitter, and other) that contributed to the total intensity rating. Subjects rinsed with distilled water and waited 30 seconds after each stimulus. Analysis of the data revealed that there was general suppression of sodium chloride taste intensity by all of the odorants, as well as other specific tastant/odorant interactions. Several competing hypotheses concerning the nature of taste modification by odorants will be discussed in light of these results.

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The Enhancement of Sweetness by Strawberry Odor is Instruction-dependent. ROBERT A. FRANK, NANCY WESSEL & GREGORY SHAFFER (Univ. of Cincinnati)

We have previously reported that some odors, but not others, enhance the sweetness of stimuli containing sucrose (e.g., Frank & Byram, 1988, Chem. Senses, 13:445-455). In those experiments, subjects were instructed to ignore all of the attributes of the stimuli except sweetness. In Experiment #1, subjects were asked to make judgments of total stimulus intensity, and then to partition total intensity into ratings of sweet, salty, sour, bitter and/or other tastes. Subjects assessed factorial mixtures of sucrose (0.1, 0.25, 0.5 M) and natural strawberry flavor (0.25, 0.5, 1.0%) plus the unmixed components and distilled water. The stimuli consisted of aqueous solutions (volume = 3.0 ml) which were swallowed by the subjects. The stimuli were rated on a 21 point category scale, and subjects were given sample stimuli to anchor the response scale prior to testing. Following each stimulus, subjects rinsed with distilled water. A 30 sec ITI was used. RESULTS - Although strawberry odor added to the total intensity of the stimuli, it did not add significantly to sweetness ratings of the solutions. Experiment #2 was an replication of Experiment #1 except that subjects made sweetness ratings only. The instructions emphasized that the subjects should ignore all other attributes of the stimuli. RESULTS - Sweetness ratings were significantly enhanced by the strawberry odorant. CONCLUSIONS -The results of these studies clearly demonstrate that subjects can treat taste/smell mixtures in an analytic or synthetic fashion depending on the instructional set they are given. These results and additional data from our lab suggest that the extent to which subjects act in an analytic vs. synthetic manner is dependent on instructions and the sensory properties of the taste/smell mixtures they are asked to evaluate.

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The Effect of Different Color Intensities on Color-Induced Odor Enhancement. LORI A. WHITTEN & DEBRA A. ZELLNER (Shippensburg University)

Our prior research has shown that coloring odorous solutions increases perceived odor intensity. Here we report on the influence of color intensity on degree of odor enhancement. Forty-four subjects rated the odor intensity of strawberry and mint extracts in distilled water. Four levels of color intensity were used--clear, light, medium, and dark (red for strawberry, green for mint)--to color one odorant concentration (3% for both strawberry and mint). Ratings of strawberry odor peaked at the medium color intensity. For mint, odor ratings increased monotonically with color intensity, and the ratings for dark green significantly exceeded all the others. These results suggest that for some odors color-induced odor enhancement increases with increasing color intensity (e.g., mint) but for others it does not (e.g., strawberry). Perhaps for the second type of odor, color appropriateness matters more than color intensity.

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Influence of Acid, Salt, and Fat on the Perception of Saltiness, Sourness, and Selected Texture Parameters of a Cheese Analog. C.R. STAMPANONI (University California Davis), R.M. PANGBORN (University California Davis), and A.C. NOBLE (University California Davis).

Twelve cheese analogs varying in amounts of fat (10, 17.5, and 25%), sodium chloride (0.5 and 2.0%), and citric acid (0.1 and 1.2%) were prepared, using a 3x2x2 factorial design. Sourness, saltiness, springiness, firmness, cohesiveness, and adhesiveness were evaluated on graphic scales. The dynamic changes over time of sourness, saltiness, and firmness were also studied for 8 cheese analogs (2x2x2 factorial design) using a time-intensity procedure. Compression and puncture tests were adopted for the instrumental measurement of the texture related attributes. Cylinders of cheese were utilized for the evaluation of sensory attributes and for instrumental measurements. Raising salt content increased maximum intensity and total duration of saltiness and sourness. Saltiness perception was not significantly enhanced by acid addition. Salt addition yielded lower springiness, and cohesiveness ratings while increasing perceived firmness. Higher acid content, in addition to increasing sourness and firmness maximum intensity, significantly decreased cohesiveness, adhesiveness, and springiness. Fat primarily influenced texture perception: higher fat contents resulted in a softer, less springy, but more cohesive and adhesive cheese analog, while saltiness maximum intensity was not modified by fat and sourness was lowest at the lowest fat level. Temporal changes in saltiness, sourness, and firmness perception upon mastication will be also discussed.

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Making the Taste of Salt More Detectable in Forced Choice Procedures: Implications for Sensory Difference Tests. MICHAEL O'MAHONY (Dept. Food Science and Technology, University of California, Davis.

Sensory difference tests form a vital part of the sensory evaluation of food. They vary in their sensitivity and this variation has been demonstrated to depend on the clarity and ability to recognise input from each of the stimuli in the test. The clarity of input, in turn, depends on the sequence of tasting of the stimuli in the test. This forms the basis for a predictive tool called Sequential Sensitivity Analysis. The basis of this is that a test with more favorable sequences of the stimuli will render the subject more sensitive to differences. The tool has been applied to beverage systems. However, much work has been applied to a model system of low concentration of NaCl and water. Here, the detectability of NaCl varies depending on whether it was tasted after a prior water or NaCl stimulus. In the same way, the detectability of water varies depending on whether it followed a water or NaCl stimulus. There is a distinct order of detectability for these four sequences and these can be used to predict success in various forced choice procedures. The detectabilities them-selves depend on adaptation, learning, interference with the stimulus by saliva and differences in supra- and subadapting sensitivity. For this NaCl/water system, the model makes specific predictions that a paired comparison test will render subjects more sensitive to differences than a duo-trio test, while a duo-trio test with only water as the standard would be intermediate in sensitivity. This prediction was confirmed using a mixed related and unrelated samples design. The paired comparison test generally requires that the nature of the taste quality difference be specified but this was circumvented by using a warm-up procedure. The A-Not A test was also examined, although it had to be modified with sureness ratings to counter response bias and allow a signal detection analysis.

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Flavour Enhancing Properties of Talin
A. F. Bingham and G. G. Birch (Reading University, Reading, U. K.).

Physicochemical and sensory studies were performed on mixtures of the protein talin with either menthol or sucrose in order to ascertain whether the enhancer molecules interact in any way with either the flavour or taste molecules. Time-intensity evaluations of such mixtures were performed. The addition of sub-gustatory threshold levels of talin resulted in the enhancement of the sweet taste and the minty flavour. The maximum intensity and total persistence time of 1 ppm menthol solution were increased, whereas, at the 10 ppm level, the addition of talin resulted in the solution possessing a greater maximum intensity. Addition of talin to solutions of 0.5, 1, 3 and 5% sucrose resulted in the mixtures possessing greater persistence times than the pure sucrose solutions. Gas chromatographic headspace analysis was performed on mixtures of menthol and talin. The addition of talin did not have a significant effect on the activity coefficent of menthol. The surface tension of sucrose-talin and menthol-talin mixtures did not differ significantly from their respective expected values. The methods of densitometry and optical rotation did not support the hypothesis that a complex between talin and sucrose was formed in solution.

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The Effect of Caffeine, Ethanol, and Sucrose on Temporal Perception of Menthol. J.M. OPET, R.M. PANGBORN, A.C. NOBLE (Univ.CA, Davis) and T.A. PERFETTI (R.J.Reynolds)

Solutions of L-menthol (150 mg/L) were presented alone and in combination with 0.20% caffeine, 8% ethanol, or 10% sucrose for evaluation of headspace by oral (inhalation) and nasal (nasal) inhalation, and of liquid orally with the nares blocked (oral) or unblocked (retronasal). Twenty subjects rated menthol intensity of the stimuli by time-intensity (T-I) methodology. Although the solutes significantly affected perception, the mode of presentation accounted for the largest difference in responses. Inhalation yielded the lowest maximum intensities (MAX), although the time to max and the duration (DUR) by inhalation was longer than that found for nasal. Responses to the liquid stimuli had higher MAX and longer times to max and DUR than those for the vapor. In comparison of responses of solutions with added solutes to those of menthol alone, when olfactory clues were removed (ORAL), menthol alone had the longest duration, sucrose depressed MAX, while ethanol increased it. For the retronasal mode, caffeine yielded the highest max and area under the T-I curve, although menthol alone again had the longest DUR. In a second experiment, menthol intensity was evaluated by T-I using a dual-delivery system in which the odorant was menthol alone, while the tastant was menthol, caffeine, ethanol, sucrose, or water. Dual delivery responses were not significantly different from retronasal perception in Expt. 1. Menthol with menthol elicited significantly higher responses than any other combination of stimuli, however two types of responses were observed. One group rated the menthol with menthol far higher in all parameters than the rest of the combinations of stimuli which weren't discriminated. For the second group, who rated the menthol with menthol condition highest, menthol with caffeine and menthol with ethanol had higher MAX and DUR than menthol with sucrose.

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Molecular Mixture Models. Daniel M. Ennis (Philip Morris Research Center, P.O. Box 26583, Richmond, VA 23261.

This paper extends the binary mixture model previously published by Ennis (1989) which modelled the relationship between mixture components on the basis of cooperative binding between stimulant molecules and a common receptor. In this paper, models which involve two new features are derived: a) independent receptors with simple binding by stimulant molecules (one-to-one binding), and b) the simulant-receptor complex reacts further with a third substance and this new complex is directly related to the percept. These new models distinguish between two important properties of odorants and tastants: their *affinity* in binding to receptors and their *efficacy* in producing a particular sensation. Parameters which model these properties can be estimated. Several generalizations can be drawn from these new models: a) if a common receptor for two substances exists and if simple binding to the receptor occurs, there will be a linear relationship between the components of mixtures which have equal perceptual effects, even following an additional transduction step prior to perception; b) if cooperative binding to a common receptor occurs, the relationship between mixture components will be nonlinear, and c) if simple binding to independent receptors occurs, the relationship between mixture components may be nonlinear, and similar to b). These models were evaluated using the data of De Graaf and Frijters (1986) on the perceived sweetness of binary mixtures of glucose and fructose. The results of this analysis revealed that if a common receptor occurs for these two compounds, cooperative binding must also exist. If simple binding occurs, then independent receptors and the participation of a third binding entity are required to model the data.

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The Effect of Ambient Odor on Creativity.
SUSAN C. KNASKO (Monell Chemical Senses Center)

Due to the association of olfaction and creativity to similar right brain functions, it was hypothesized that creativity tasks might be a type of performance easily influenced by ambient odor. Ninety subjects individually participated in two one-hour sessions that were one week apart. In one of the sessions the testing room was scented while in the other it was not scented. The order of the odor presentation was randomized. Thirty subjects (15 women and 15 men) were in each of the three odor conditions: lemon, lavender, or dimethyl sulfide. During both sessions subjects completed creativity tasks as well as questionnaires concerning their mood, health and perception of the testing environment. Personality questionnaires concerning: locus of control, arousal seeking tendency, level of extroversion and information processing were also completed.

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Dose-Related Effects of Cigarette Smoking on Olfactory Function. RICHARD E. FRYE, RICHARD L. DOTY, (Smell and Taste Center, and Department of Otorhinolaryngology and Human Communication, University of Pennsylvania, Philadelphia, Pa.) BRAIN S. SCHWARTZ (Clinical Epidemiology Unit, and Section of General Internal Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pa).*

Little is known about the influence of cigarette smoking on the ability to smell; previous studies on this topic have led to contradictory findings and have failed to take into account smoking dose and duration. In the present study, the 40-odorant University of Pennsylvania Smell Identification Test (UPSIT) was administered to 638 subjects for whom detailed smoking his-tories were available. Smoking was found to be adversely associated with odor identification ability in a dose-related manner in both current and previous cigarette smokers. In previous smokers, improvement in olfactory function was related to the time elapsed since the cessation of smoking. Logistic regression analysis found current smokers to be nearly twice as likely to evidence an olfactory deficit (i.e., UPSIT scores less than or equal to the tenth percentile) than persons who have never smoked. Overall, the data suggest that (a) smoking causes long-term but reversible adverse effects on the ability to smell and (b) the failure of some studies to demonstrate smoking effects may be due to the inclusion of persons with a past history of smoking in the non-smoking groups.

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Analysis of Trial Sequence Position and Diluent Type on the Single Staircase Odor Detection Threshold Value for Phenyl Ethyl Alcohol. JOHN D. PIERCE, JR., UDAYAN AGRAWAL, RICHARD L. DOTY (Smell and Taste Center, and Department of Otorhinolaryngology and Human Communication, University of Pennsylvania, Philadelphia, Pa.)*

Although odor detection thresholds are commonly measured in the clinical assessment of olfactory function, procedural factors affect the threshold value. The present study evaluated the influences of (a) trial sequence position and (b) diluent type on the threshold value for the rose-like odorant phenyl ethyl alcohol (PEA). Twenty adult subjects were administered two ascending single staircase forced-choice odor detection threshold tests using PEA diluted in either propylene glycol or light USP grade mineral oil. The tests were presented successively and in counterbalanced order. Threshold values were significantly influenced by diluent type (lover for mineral oil) and trial sequence (lover for later odorant presentations). These findings emphasize the need for standardization of threshold procedures and imply that a single series ascending method of limits procedure likely underestimates the degree of olfactory sensitivity.

*Supported by National Institute on Deafness and Other Communication Disorders Grant P01 00161.

The Relationship Between Nasal Anatomy and Uninasal Human Olfaction. DE Hornung, DA Leopold, PR Sheehe, SL Youngentob, MM Mozell (Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, New York, USA)

The relationship between uninasal anatomy and uninasal olfactory ability was evaluated by correlating measurements from nasal cavity CT scans of 18 patients with the results of an established clinical measure of olfactory function (the Odorant Confusion Matrix or OCM). For each patient, each of the two nasal cavities was divided into 21 compartments. The these compartments, both alone and in various combinations, were used as possible independent variables in a stepwise analysis of variance in which the logit of the uninasal OCM score was used as the dependent variable. The final model contained 6 individual compartments and 9 combinations of compartments which together accounted for over 95% of the variability seen in the uninasal OCM score. These compartments which make up the model are for the most part anterior and inferior to the olfactory cleft and generally, as they increase in volume, the performance on the OCM improves. The establishment of this relationship suggests that the detailed shape of the nasal airflow pathway should also be considered in the clinical evaluation of patients with olfactory dysfunction.

Supported by NIH Grant #DC0020

The Insect Behavioral Response to Sex Pheromone Is Inflected near Threshold: Evidence of Feature Detection? R.W. MANKIN (Insect Attractants, Behavior, and Basic Biology Research Laboratory, US Dept. Agric.-ARS, Gainesville, FL)*

The proportion of male moths that take flight tends to increase as the stimulating concentration of sex pheromone increases. The pattern of this stimulus-response curve is considerably different from the response pattern of antennal pheromone receptor neurons to the major sex pheromone component. On a standard psychophysical, log-log plot of response vs. stimulus intensity, the best fit to the behavioral relationship is given by two separate straight-line segments connected at a point of inflection, and the best fit to the neural response is a single line segment. The point of inflection lies at a concentration an order of magnitude above the neural threshold to the major sex pheromone component. This indicates that the insects detect pheromone well before they respond behaviorally. Apparently, a central integration process occurs which requires higher stimulus input than that provided at the peripheral neural threshold. Such a process may contribute to the behavioral discrimination of pheromone blend that is exhibited by many insects at long distances downwind from a pheromone source.

*I wish to acknowledge M. S. Mayer, Alan Grant, P. Wilkening, and J. Sharp, who contributed valuable assistance to this research.

Peptide and Amino Acid Mimics of Crab Pumping Pheromones. DAN RITTSCHOF, RICHARD B. FORWARD, JR., CARRIE U. BUSWELL AND D. MATT WACHOWIAK (Duke University Marine Laboratory).

Most species of brachyuran crabs release larvae synchronously and at a time related to environmental cycles such as time of day, time of tide and phase of the moon. Synchrony is orchestrated by pheromones released from hatching eggs that act upon the female. Embryos undergo development in eggs glued by the female to hair-like structures on her abdomen. Exposure to pheromone causes the ovigerous female crab to perform a stereotypic series of larval release behaviors in which she vigorously pumps her abdomen. The pumping of the abdomen serves to help rupture unhatched eggs and results in hatching synchrony. We have demonstrated indirectly that some of the pumping pheromones are neutral-basic peptides.

Here we examine the potency of the structural classes of L-amino acids and of di- and tripeptides. Of the amino acids, those with large hydrophobic side chains are most potent with approximately picomolar thresholds. Of the peptides, tripeptides are most potent. The most potent tripeptide was GIR which was effective at approximately attomolar concentrations. Response thresholds were observed to increase 6 orders of magnitude with GIR, IGR, GLR and LGR. From these and other data on potency of free amino acids and peptide analogues, we conclude that the hydrophobic region associated with the second amino acid in the tripeptide is important both in binding and in transduction of the response.

Effect of Stimulus Intensity on Discrimination of Odorant Mixture Quality by Spiny Lobsters in an Associative Learning Paradigm. J.B. FINE-LEVY AND C.D. DERBY (Dept. of Biology, Georgia State University, Atlanta, GA)

Previously, we have shown that the Florida spiny lobster, *Panulirus argus*, can behaviorally discriminate between members of a set of four artificial odorant mixtures: crab, mullet, oyster, and shrimp. In our previous paradigm, each group of lobsters tested was conditioned to avoid two concentrations (0.05 and 0.5 mM) of the designated conditioned mixture in order that they attend to stimulus *type* rather than concentration. They were then tested with the same two concentrations of each of the nonconditioned stimuli. In contrast, the present experiments were designed to examine the effects of mixture intensity on inter- as well as intra-type mixture discrimination. This was accomplished by conditioning lobsters to only one concentration (0.5 mM) of shrimp mixture and testing them with three concentrations (0.005, 0.05, and 5.0 mM) of shrimp mixture and two concentrations (0.05 and 0.5 mM) of oyster mixture. For the two appetitive individual behaviors examined, lobsters were unable to discriminate the conditioned mixture (0.5 mM shrimp) from any other of the same-type nonconditioned mixtures. For a third behavior, active avoidance, lobsters discriminated, to a significant degree, the 0.5 mM shrimp mixture from all of the nonconditioned mixtures (0.005, 0.05, 5.0 mM shrimp; 0.05 and 0.5 mM oyster). However, aversion values for the nonconditioned shrimp mixtures were consistently and markedly higher than those for the nonconditioned oyster mixtures for all three behaviors. Thus, when spiny lobsters are forced to use intensity as a cue (as in an aversive conditioning situation), they have the *ability* to discriminate between mixtures of the same type but different intensity. However, based on examination of postconditioning decreases in appetitive behaviors, it is most likely that mixture intensity has a relatively minor effect on discrimination of odorant mixture type by these animals, at least over a 1000-fold concentration range.

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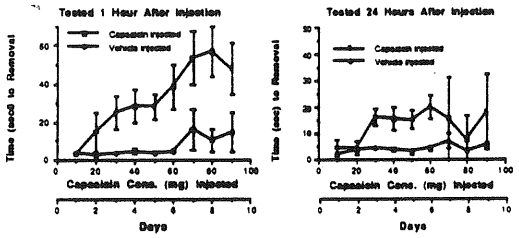
The Effect of Capsaicin Treatment on Tiger Salamander Responses to Chemical Irritation. C.C. KEELEY, W.L. SILVER. Dept. of Biology, Wake Forest Univ.,Winston-Salem, N.C. 27109.

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 mammals produces an insensitivity to noxious chemical stimuli, apparently due to the depletion of substance P, or other neuropeptides, from nociceptive fibers. Capsaicin appears to have little effect on birds and a small effect on frogs. In the present experiment, capsaicin was injected into land-phase tiger salamanders in increasing concentrations (10, 20, 30, 40, 50, 60, 70 ,80, 90, mg/kg), over a period of 9 days. The animals' responses to chemical irritants were examined each day 1 hr and 24 hrs after injection by measuring the time it took to remove a piece of filter paper saturated with HCl (0.05 or 0.1M) placed on the skin bridging the nares. Controls (injected with vehicle) responded to 0.05M HCl 1 hr after injection in 2 to 27 s and 0.1M in ≤ 2 s. Capsaicin-treated salamanders, responded to 0.05M 1 hr after injection significantly slower than did controls with latencies increasing over the 9 test days. There was no significant difference between the latencies of the responses to 0.5M HCl of the capsaicin treated animals and the controls tested 24 hrs after injection. All of the capsaicin-treated animals as well as the controls responded to 0.1M in less than 3 s on each day. These results suggest that capsaicin may have a small effect on the nociceptors of salamanders.



Supported by NIH Grant No. NS-23326.

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Effects of Deoxycorticosterone Acetate (DOCA) on Salt and Water Intake and Metabolism in Rats. ERKADIUS, R. A. BERNARD and K. J. MOONEY (Dept. of Physiology, Michigan State University, East Lansing, MI 48824)

Two groups of male Sprague Dawley rats (n=10) were used. The experimental group was injected with DOCA (5mg/kg/12h s.c.) and the controls received an equal amount of sesame oil. The animals were housed individually in plexiglass metabolic cages fitted with two drinking bottles. Standard rat chow (Teklad ™, powdered) containing 0.19 meq Na/gm was offered ad libitum in a removable feed drawer. Preference tests were begun 19 days after the start of DOCA injections, which continued throughout the experiment. The 19 days served as an adaptation period to allow DOCA to reach a steady state. NaCl solutions were presented in order of increasing concentration, from 0.001 to 1.0 M, each for a two-day period. Distilled water was presented in both drinking bottles during the adaptation period. From the temporal relationships in these experiments we found that DOCA administration first reduced Na excretion, which was followed by escape on the second day, and increased urinary output on the same day. Water intake increased on the first day but was not significantly different from control until the 9th day when it reached a plateau. Urinary output was significantly elevated from the 2nd day and was approximately twice that of the controls by the 10th day and thereafter. Taste preference threshold for NaCl was less than 0.001 M compared to 0.03 for the controls and highest preferred concentration was 0.3 M compared to 0.1 M for the controls. Volume intake of the salt solutions was 6 times greater than control at 0.1 and 0.15 M and the quantity of Na ingested in the solutions was 8 times greater than control at 0.15, 0.2 and 0.3 M. These increases were accompanied by increased Na excretion and urine volume. We conclude that DOCA increases salt appetite by decreasing taste preference threshold and increasing the acceptance for higher concentrations of NaCl.

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A Computerized Method for the Determination of Odor Detection Thresholds in Mice. CHRISTOPHER M. PALATUCCI and ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, USA)[1]

The accurate determination of psychophysical thresholds and quality comparisons among olfactory stimuli has long been complicated by a variety of instrumental and behavioral factors. Among these are deficiencies in odor presentation and quantification, and the design and suitability of the instrumental task to be performed by the experimental animal. We now present a method for the accurate assessment of psychophysical parameters associated with chemical stimuli including detection thresholds and odor quality identifications. The apparatus is based on the radial arm maze first described by Olton & Samuelson (*J. Exp. Psych.:Anim. Behav. Procs.* 2:97-116, 1976). Water deprived animals are first trained to associate selected odor stimuli with particular arms of the maze by rewarding the correct pairing of stimulus and response with a measured amount of water. A lickometer circuit connected to a port in the floor of each arm controls a water valve. Licking the port to activate water delivery represents an operant which is not aversive to the animal and is well within its behavioral repertoire. Incorrect responses are signaled by licking the port in the wrong arm. In a test session, odor flow is initiated by the subject breaking a photoelectric beam in the start arm of the maze. Animals sample the odorant by inserting their snouts into a port at the end of this arm for a specified period of time. They then indicate their decision concerning odor quantity or quality by licking a water port in one of the arms. The entire process is controlled by a microcomputer running a "C" program. In order to test for response bias the entire maze is constructed of interchangeable modules. Threshold data will be presented.

[1] We thank Drs. M. Mozell, and S. Youngentaub for their advice and R. Sypek for programming assistance. Supported by NIDCD grants DC00131 & DC00371 to RJO.

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A Detailed Comparison of Sucrose and Saccharin Drinking by the Laboratory Rat. JAMES C. SMITH (The Florida State University).

In this laboratory we have developed a system for studying the details of how rats ingest foods and drink various tastants in a normal home cage environment. Rats ingest foods and fluids in discrete episodes called bouts. We are able to measure the number and duration of these bouts, day-night distributions of bouts, rate of ingestion during bouts and the temporal relations between eating and drinking bouts. Such measures have been recently reported for a variety of sucrose concentrations, but only one saccharin concentration has been studied. In the present study the patterns of ingestion of a variety of palatable concentrations of saccharin and sucrose are compared. Seven concentrations of saccharin ranging from .001M to .064M were compared to sucrose concentrations ranging from .03M to 2.0M. As expected, food intake did not change with increased saccharin concentrations, but did show a marked decrease as sucrose concentration increased. As concentration of the two solutions was systematically increased, marked differences were observed in the patterns of ingestion between sucrose and saccharin. The number of night drinking bouts decreased for both sucrose and saccharin while the number of day bouts remained fairly constant. Both bout duration and number of licks per bout were quite different for the sucrose and saccharin solutions. Switches from food to sucrose decreased in a quite systematic manner but there was no change with saccharin. Rate of sucrose licking during a bout showed orderly increases but not with saccharin. Many other dependent variables are compared.

Supported by AG04932 and AG06841

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A moment-by-moment comparison of intake of five bitter compounds by Sprague-Dawley rats. JOHN I. GLENDINNING & JAMES C. SMITH (Florida State University)

We explored how bitter compounds influence drinking patterns in Sprague-Dawley rats. The compounds were quinine HCL (QHCL), sucrose octa-acetate (SOA), caffeine, denatonium benzoate (DB) and *n*-propyl-6-thiourea (PROP). Eight males were each subjected to 4 ascending series of concentrations of each compound in single-bottle free-licking tests. During each test, individual rats received a bottle containing the test solution for 23 h, and then a new bottle containing only water for the next 24-48 h. Tests were conducted in cages equipped with a lickometer device that calculated 1) duration of each drinking burst and 2) lick rate during bursts. To minimize post-ingestional and adaptation effects, we limited our analysis to those bursts that occurred while rats drank the first 2 ml of solution in each test. Burst duration and lick rate decreased linearly with increasing concentrations of QHCL, DB and PROP. In contrast, burst duration and lick rate values were significantly higher than water baseline values at low concentrations of SOA and caffeine, and lower than water baseline values at higher concentrations. We also compared the sensitivity of the response variables, burst duration and lick rate, to varying concentrations of each compound. Lick rate appeared to be more sensitive than burst duration at low concentrations. We conclude that 1) our single choice paradigm offers a powerful tool for examining how rats ingest bitter solutions, and 2) rats become progressively more averse to increasing concentrations of QHCL, DB and PROP, 3) rats show a preference-aversion response to increasing concentrations of SOA and caffeine, and 4) lick rate is more robust than burst duration as a measure of taste aversiveness in rats.

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Conditioned Suppression as a Psychophysical Method for Taste Threshold Determination. A. KURT THAW (Florida State University), JAMES C. SMITH (Florida State University)

An apparatus was designed to determine taste thresholds in the rat utilizing a conditioned suppression procedure. Animals were reduced to 85% body weight and trained to lick a sipper tube through a slot in the back of an experimental chamber for pellet reinforcement. Any one of eight seperate drinking tubes was presented for each trial. Animals were trained on a fixed ratio schedule and then placed on a variable interval (VI) schedule for testing. While on the VI schedule subjects were trained to suppress licking behavior to any tastant other than water. A mild electric shock was administered if the rat made more than 20 licks on a non-water tube within a 10 second time period. The criterion for detection of a substance was the animal's behavior toward the solutions presented, i.e. detected solutions did not result in 20 or more licks. Thresholds have been measured on a variety of taste compounds and these values have been compared to those taken by other behavioral procedures.

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Influence of the D-1 Dopamine Receptor Agonist SKF 38393 on the Odor Detection Performance of Male and Female Rats. CHENG LI, CHERYL A. PFEIFFER, JUDITH M. RISSER, and RICHARD L. DOTY (Smell and Taste Center, School of Medicine, University of Pennslyvania, Philadelphia, PA)*

Previous research has shown that the D-2 dopamine receptor agonist quinpirole decreases the odor detection performance of rats (Doty and Risser, Psychopharmacology 98:310-315, 1989). In the present study, the effects of the selective D-1 receptor agonist SKF 38393 (1.0, 2.5, 5.0, 7.5, 10.0, 12.5 mg/kg SC) on the odor detection performance of 18 adult male Long-Evans rats was assessed using high precision olfactometry and a go/no-go operant signal detection task. The drug doses and a saline control condition were administered every third day, in counterbalanced order, with injections occurring 20 minutes before each daily 260-trial test session. Relative to saline, odor detection performance to ethyl acetate was significantly enhanced at SKF 38393 doses 7.5, 10.0, and 12.5 mg/kg ($p < .001$). The odor detection performance of 6 adult female Long-Evans rats to eugenol was similarly enhanced by this drug. Prior administration of SCH 23390 resulted in motoric disturbance, even at low doses, precluding a determination as to whether this effect could be mitigated by a D-1 receptor antagonist. These data suggest that odor detection performance of rats can be enhanced by selective pharmacologic stimulation of dopamine D-1 receptors.

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Concanavalin A selectively inhibits odor detection in the rat. **R. APFELBACH[1], W.F. ASSELBERGS[2], A. NAGORNY-DEBUS[1] and E.H. POLAK[2] ([1]Dept. of Zoology, University of Tübingen, Tübingen, FRG, [2]Olfaction Research Group, Dept. of Chemistry, University of Warwick, U.K.).**

When the rat olfactory mucosa is treated with concanavalin A (Con A), it subsequently shows diminished EOG activity for many odorants. The aim of the present study was to find behavioral correlates to this earlier electrophysiological finding. Thus, male Wistar rats 150-200 days old were tested in an olfactometer for their performance in detecting low odor concentrations. Two odorants were used: Ethyl acetate (EA) as a reference, and dimethyl disulphide (DMDS) as a Con A inhibited odorant. When testing the animals psychophysical abilities, clean air and odors in concentrations above thresholds were offered randomly. When testing for EA, there were no significant differences in the odor detection ability before (92,3% correct reactions) and after (92,5% cr) Con A treatment. However, Con A seems to have a marked effect on DMDS detection since the animals were unable to distinguish between clean air and DMDS (58,6% cr). These results support the idea that Con A selectively inactivates one or more types of olfactory receptors.

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Domestic Pig: Possible Model for Study of Specific Anosmia to Androstenone. KATHLEEN M. DORRIES, ELIZABETH ADKINS-REGAN, BRUCE P. HALPERN (Cornell University, Ithaca NY 14853-7601)

The steroid 5α-androst-16-en-3-one (androstenone), a major component of boar odor which is also produced by human males, facilitates mating behavior in estrous sows. Specific anosmia to androstenone occurs in approximately 40% of human adults tested, with adult males less likely than females and prepubertal children to smell its odor (Dorries, et al., 1989). The pig is a potentially valuable animal model for the study of specific anosmia to androstenone because its response to the steroid varies with age and sex in a pattern similar to that noted in humans: Immature pigs and males castrated prepubertally, in addition to sows, will exhibit receptive behavior when primed with estradiol, but mature intact males will not (Adkins-Regan, et al., 1989). To test whether the boars' lack of behavioral response to androstenone reflects an inability to detect the chemical, three boars have been trained to respond to odors but not blanks using a "go, no-go" discrimination task. They were trained to open a reward box in the presence of the odorant geraniol, then tested with amyl acetate and androstenone. Mean number correct responses for the three pigs on the first block of 100 amyl acetate trials was 85. All three performed at chance (mean = 52 correct) in the first block of androstenone trials. The performance of two improved gradually, reaching 80% after 4 blocks, each block run on a different day. The third continued to respond at chance throughout testing. Thus preliminary results suggest boars are insensitive or initially insensitive to the odor of androstenone. Mature females and prepubertal pigs will be tested in the same manner and their sensitivity compared with that of boars to further determine if swine exhibit a pattern of specific anosmia to androstenone similar to that seen in humans.

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Micro-anatomy of the Trunk Tip of Elephas maximus

L.E.L.RASMUSSEN, (Oregon Graduate Institute)
BRYCE L. MUNGER, (Pennsylvania State University)

This study documents the characteristics of the sensory innervation and cutaneous receptors in the dermal and epidermal skin of the extreme trunk tip (finger) of the Asian elephant Elephas maximus by light and electron microscopy During the flehmen response the elephant moistens the trunk tip with liquids of interest and apparently uses this tip for the transport of such substances to the mucous-filled openings of the incisive ducts, which lead to the vomeronasal organ. We expected to find this region of the trunk tip richly innervated, perhaps with specialized nerve endings, especially in the epidermis. Unexpectedly, our light and electron microscopic examinations demonstrated three distinctive features. First, a uniquely high density of free nerve endings was apparent in the superficial layers of the trunk tip skin. Second, unusual tiny vibrissae hairs of extreme shortness surrounded by hundreds of axons were interspaced with more conventional vibrissae hairs. Third, complex and distinctive Meissner-like corpuscles were abundant, surprisingly not in the epidermis but in the superficial layer of the dermis. This study provides basic histological and cytological information about the trunk tip region prior to our projected investigation of trigeminal innervation of the trunk region and possible chemosensory receptors within the trunk orifice.

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Chemical Profiles of Temporal Gland Secretions from Captive Asian Bull Elephants During Musth and from African Bull Elephants, Living in Wild but Crowded Conditions.

L.E.L.RASMUSSEN (Oregon Graduate Institute)
D.L.HESS (Oregon Regional Primate Center)
A. HALL-MARTIN (Inland Parks, S.Africa)

This study compares the volatile components of the temporal gland secretions of captive Asian bull elephants in musth and a distinctive group of wild African bull elephants, confined to a small national park. The captive Asian population has been well studied (Rasmussen et al., 1984; Rasmussen, 1988). Serum testosterone was elevated at specific times; aggressive behaviors occurred concomitantly with temporal gland secretions, although aggression and elevated serum testosterone were not always related. Selected volatiles among the 23 compounds identified demonstrated concentration changes during the progression of musth, at times simultaneously with alterations in testosterone levels (Rasmussen et al., 1989). The African bull elephants have been monitored, behaviorally and physiologically, by radiocollared tracking and monthly sampling during the past five years. Aggressive behaviors similar to those of Asian bull elephants have been documented; serum and temporal gland testosterone were elevated concomitantly in a cyclical fashion similar to musth in Asian elephants. Chemical characterization of the volatiles of the temporal gland secretions from these bulls revealed similarities to the compounds described in Asian bulls. Several carboxylic acids, a ketone- 2-nonanone- and an alcohol- 5-nonanol- not previously described in African temporal gland secretions were identified. Our current investigations on Asian elephants demonstrate consistent avoidance by cows and young bulls to fresh musth secretions from adult bulls; perhaps some of these volatiles from the temporal gland secretions communicate information among elephants.

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Molt Control in Sexually Mature Female Lobsters. DIANE F. COWAN (Boston University Marine Program)

Behavioral observations reveal that molting in mature female lobsters is linked to reproduction in the following two ways. Firstly, mating occurs approximately 1/2 h after the female completes ecdysis. Current results support the hypothesis that the male protects the vulnerable, soft-shelled female from injury after her molt. Secondly, egg extrusion occurs approximately two months postecdysis whether the female has been inseminated or not. Therefore, the female must mate between the time of ecdysis and egg extrusion in order to avoid brooding unfertilized eggs. Lobsters experience indeterminate growth and, eventually, must molt. Varying the sex ratio in 4000 gal aquaria between May and November 1983, 1986, 1987, and 1989, resulted in significant differences in the timing of female molts. For example, in four trials using a sex ratio of 5F:2M, 20 out of 23 females molted (3 females were removed and replaced by an additional 3 females in one of the trials). All 20 of these females pair bonded, cohabited with a dominant male, and mated after molting. In addition, their molts were staggered throughout the breeding season as though each female delayed her molt until it was her turn to pair bond with the dominant male (Cowan & Atema, Anim. Behav., in press). In contrast, only 4 out of 20 females molted when a sex ratio of 5F:0M was tested. Therefore, females apparently have some control over the timing of their molting and therefore reproduction. In mammalian social systems such control over reproductive receptivity is known to be mediated by primer pheromones. Since lobsters can discriminate sex based on odors (Atema & Cowan, J. Chem. Ecol., 1986), chemical communication may be similarly involved in this system. In summary, when a dominant male was present, female lobsters molted and when no male was present they generally did not. Perhaps male odor has a priming effect that results in acceleration of the female molt cycle and perhaps female primer pheromones act to delay molting. This would explain both molt staggering and molt delay.

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Characterization of a *Paramecium* Ca^{2+}-ATPase: putative transduction component. M. V. WRIGHT and J. VAN HOUTEN (University of Vermont, Burlington, Vt. 05405)

Previously we found that Ca^{2+} efflux may be involved in signal transduction in Paramecium. To further investigate the possible mechanisms through which Ca^{2+} extrusion may function in chemoreception we have isolated a Ca^{2+}-ATPase activity. Here we report the characteristics of this activity that implicate its role as a plasma membrane pump. The Ca^{2+}-ATPase activity requires 3 mM Mg for optimal Ca^{2+} stimulation (K_{Ca}=90 nM) and is specific for ATP as substrate (K_m=75 uM). Vanadate and calmidazolium inhibit Ca^{2+} stimulated activity with an EC_{50} of 2 uM and 0.5 uM respectively. Likewise, 10 uM trifluoperazine inhibits 80% of Ca^{2+}-ATPase activity, but bovine brain calmodulin failed to stimulate. The Ca^{2+}-ATPase is not inhibited by NaAzide (10 mM), oligomycin (10ug/ml) or oubain (0.2 mM). Incubation of pellicles with [γ-^{32}P]-ATP specifically labels a 133 kDa protein in a Ca^{2+} dependent, hydroxylamine sensitive manner, and the level of phosphorylation is increased by 100 uM La^{3+}. Phosphorylation of an endoplasmic reticulum enriched fraction labels a Ca^{2+} dependent phosphoprotein different than the pellicle protein, being lower in molecular mass and unaffected by La^{3+}. Ca^{2+} uptake by the alveolar sacs, an integral component of the pellicle membrane complex, is poorly coupled to Ca^{2+} stimulated ATP hydrolysis (< 0.2) and is much less sensitive to vanadate inhibition. Therefore, the major Ca^{2+}-ATPase activity is likely to be plasma membrane associated.

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Distribution of Ca^{2+}-ATPase Activity in the Olfactory Rosette of Atlantic Salmon: Comparison with Na^+, K^+-ATPase and Alanine Receptors. Y.H. LO, T.M. BRADLEY AND D.E. RHOADS (University of Rhode Island, Kingston, RI 02881)

Evidence that Ca^{2+} may serve multiple roles in olfactory reception has prompted an investigation of potential mechanisms for controlling free Ca^{2+} levels. We have studied the presence, subcellular localization and regulation of Ca^{2+}-stimulated ATP phosphohydrolase (Ca^{2+}-ATPase) in olfactory rosettes from Atlantic salmon (Salmo salar). ATPase activity was determined as release of inorganic phosphate due to ATP hydrolysis. Cilia were isolated as the source of dendritic membranes of olfactory receptor cells and were shown to contain Ca^{2+}-ATPase activity at a level eight-fold higher than that of membranes prepared from the deciliated olfactory rosette (Rhoads et al., J. Cell Biol. 109, 254a, 1989). The activity present in the latter preparation was associated with a microsomal fraction presumably derived from nonsensory as well as sensory cells. Ca^{2+}-ATPase activity was increased in a dose-dependent manner by calmodulin which increased the apparent affinity of the enzyme for Ca^{2+}. Ouabain sensitive Na^+, K^+-ATPase activity was also present in olfactory cilia and in the microsomal fraction from the deciliated rosette. In contrast to Ca^{2+}-ATPase, the Na^+, K^+-ATPase activity of the ciliary membrane was only two-fold greater than that of the deciliated rosette. This distribution of Na^+, K^+-ATPase activity, a plasma membrane marker, was similar to the distribution of alanine binding sites, a marker for olfactory receptors in Atlantic salmon. These results have led to the working hypothesis that cilia provide increased surface area for receptor cell plasma membrane over which Na^+, K^+-ATPase and olfactory receptors are distributed, but provide a relatively specialized site for removal of Ca^{2+} from olfactory receptor cells.

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Activation of Olfactory Adenylate Cyclase by Calcium via Calmodulin. ROBERT R. H. ANHOLT, ANN M. RIVERS (Department of Neurobiology, Box 3209, Duke University Medical Center, Durham, NC 27710).*

Chemosensory cilia of olfactory receptor neurons contain an adenylate cyclase, which is stimulated by high concentrations of odorants. Cyclic AMP produced by this enzyme has been proposed to play a role in olfactory transduction. We found that olfactory cilia contain calmodulin (1.35 ± 0.36 (n=5) µg/mg protein) and that calmodulin potently stimulates olfactory adenylate cyclase (EC_{50} = 5.6 ± 0.9 µM) by a mechanism additive to and independent from stimulation by odorants. Moreover, maximal activation of the enzyme by calmodulin is 3-6 times greater than that observed with a mixture of odorants. Calmodulin-mediated activation of the olfactory adenylate cyclase is dependent on calcium. Estimates of the concentrations of free calcium in EGTA-buffered solutions show that an intraciliary increase in calcium concentration from approximately 0.1 µM to about 2 µM will lead to calcium/calmodulin mediated activation of the olfactory adenylate cyclase. Calcium/calmodulin mediated activation of adenylate cyclase is enhanced by GTP. Examination of this GTP-dependence reveals a GTP-dependent and a GTP-independent component of adenylate cyclase activation by calcium/calmodulin. These observations underscore the importance of crosstalk between second messenger systems in olfaction and indicate that olfactory transduction may be accompanied by a second messenger cascade, in which an odorant induced increase in intracellular calcium leads to activation of adenylate cyclase by calmodulin.

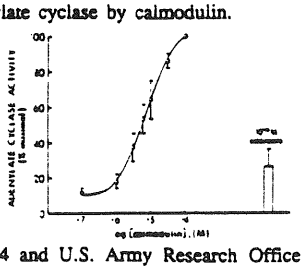

Figure 1: *Activation of olfactory adenylate cyclase by calmodulin in the presence of 0.1 mM GTP and endogenous calcium.*

* Supported by NIH grant DC00394 and U.S. Army Research Office grant DAAL03-89-K-0178.

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Inositol-1,4,5-trisphosphate (IP_3): An Alternate Second Messenger For Olfactory Transduction? JOHN H. TEETER, TAUFIQUL HUQUE and DIEGO RESTREPO (Monell Chemical Senses Center, Philadelphia, PA)

As in other animal models, stimulation of channel catfish (Ictalurus punctatus) olfactory receptors leads to a G-protein-mediated increase in intraciliary cAMP. This increase, which occurs only at relatively high stimulus concentration, may lead to depolarization through the opening of cAMP-gated channels present in the ciliary membrane of catfish olfactory neurons (Bruch and Teeter, In: Chemical Senses: Receptor Events and Transduction in Taste and Olfaction; Brand, Teeter, Cagan and Kare eds, Marcel Dekker, NY pp283-298, 1989). In addition, L-amino acids, which interact with specific olfactory receptors in catfish, stimulate rapid hydrolysis of phosphatidylinositol-4,5-bisphosphate to IP_3 in isolated olfactory cilia followed by rapid metabolism of IP_3 to other inositol phosphates, and in a subgroup of isolated olfactory cells, stimulation triggers a rapid increase in intracellular calcium. The formation of IP_3 and the increase in $[Ca_i]$ are also activated by AlF_4^- and guanine nucleotides suggesting G-protein mediation of the response. Both G-protein- and stimulus-mediated elevation of $[Ca_i]$ are the result of an influx of extracellular calcium which is not inhibited by the L-type calcium channel blocker nimodipine. We have identified a calcium channel, in phospholipid bilayers into which cilia membrane vesicles have been incorporated, which appears to be gated by IP_3 (1-10 uM) and is not inhibited by nimodipine. This channel has a slope conductance in 55 mM barium of 79±5 pS (mean±SEM, n=5) and is selective for Ba^{2+} (and Ca^{2+}) over K^+ and N-methyl-D-glucamine (minimum permeability ratios for Ba^{2+} over K^+ and $NMDG^+$ are 7 and 54 fold respectively). Calcium influx carried by these channels could cause receptor cell depolarization, suggesting that G-protein-linked phosphoinositide turnover may play a mediatory role in olfactory transduction through the opening of an IP_3-gated calcium channel.

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Structural Features of Ventral Chemosensory Organs in Scorpions and Solpugids Suggest Common Evolutionary Origin. PHILIP H. BROWNELL (Oregon State University, Corvallis, OR 97331-2914)

The pectines of scorpions and the malleoli of solpugids (Class Arachnida) are large, chemosensory appendages of uncertain function. The structure and arrangement of sensilla on these organs indicate they are used to taste or smell the substrate during locomotion. Our behavioral and physiological studies support this hypothesis and further indicate that male scorpions use the pectines to locate conspecific females by detection of a pheromone. Although the pectines and malleoli look very different externally, they show striking similarities in sensillar morphology and the projections of chemosensory afferents to the CNS. In both systems the sensillar pore openings are slits with similar fine structure and orientation perpendicular to the direction of locomotion. The unit of sensory innervation in each organ is a bundle of 10 to 20 dendrites wrapped by microvillar and sheath cells that isolate the outer segments from the hemolymph space. In the pectine, five of the bundled dendrites are identifiable by their arrangement and orientation relative to the slit opening. In the central nervous system the axons of pectine and malleolar chemoreceptor cells terminate in a distinct ventromedial neuropil which is identifiable by its staining properties. These observations suggest that the ventroposterior chemoreceptor organs of scorpions and solpugids have evolved from homologous structures perhaps of common ancestry. They also fortify the view that the pectines and maleoli are used for similar purposes, namely, for detection and orientation to chemosensory stimuli located on the substrate.

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Bilateral Chorda Tympani Transection Causes Severe Impairments in the Rat's Ability to Discriminate NaCl from KCl. ALAN C. SPECTOR, DAVID DE LANGE, TAKESHI KASAGI, CHRISTINE A. KORNET, and HARVEY J. GRILL (University of Pennsylvania)

Electrophysiological evidence suggests that the chorda tympani nerve provides a major contribution to the neural coding of sodium containing compounds. A growing body of behavioral data have imparted a functional significance to these electrophysiological findings. For example, bilateral section of the rat's chorda tympani nerve (CTX) raises the NaCl detection threshold by about 2 orders of magnitude, whereas it only marginally affects the detection threshold for sucrose. The present study employed an animal psychophysical technique to examine whether the rats ability to discriminate a sodium salt (NaCl) from a non-sodium salt (KCl) is affected by CTX. Four water-deprived intact male Sprague-Dawley rats were trained to maintain licking on a drinking spout in a specially-designed gustometer to receive fluid rewards (S-). For half of the rats the fluid was NaCl; for the other half it was KCl. Three concentrations (.05, .1, .2 M) were randomly delivered during training. After rats were performing asymptotically, trials with the same three concentrations of a second taste stimulus (S+) were included. The S+ was KCl for half of the rats (S-=NaCl) and NaCl for the other half (S-=KCl). Rats had to lick the dry spout 30 times on the average (VI-30) to receive 5 sec access to a taste stimulus (taste trial). On S+ trials, if the animal did not completely suppress licking during the latter 3 sec of the trial (avoidance period), it received a mild footshock. On S- trials, if the rat did not lick during the avoidance period it received a 30 sec time-out which delayed the presentation of the next fluid delivery. Rats generally completed 100-200 trials during their 60 min sessions. After 10 days of discrimination training, the average number of avoidance period licks to the S+ collapsed across concentration was 1.0 and to the S- was 15.1. Two experiments followed. First, rats were tested for generalization to a variety of solutions. Rats generalized their NaCl responses to lithium- or sodium-containing salts (LiCl and MSG). In contrast they generalized their KCl responses to non-sodium salts ($MgCl_2$, $CaCl_2$, NH_4Cl, and monoammonium glutamate). Water and 0.3 M sucrose produced responses in between that observed for NaCl and KCl. Second, the effect of CTX on the NaCl vs. KCl discrimination was examined. To summarize the basic finding, prior to CTX in rats, the average difference between their avoidance period licks to the S+ and S- (collapsed) was 14.3. In contrast, following CTX this difference was 2.6. Statistically, the rats still demonstrated some degree of discriminability between NaCl and KCl following CTX, although the extensive overlap of the response distributions to the two compounds demonstrates a marked impairment in this ability. To study the taste specificity of this effect, we are currently testing rats in a similar way for their ability to discriminate sucrose from quinine, before and after CTX.

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Removal of the Vomeronasal Organ Inhibits Reproductive Physiology and Behavior in Female Prairie Voles. JOHN J. LEPRI (Department of Biology, University of North Carolina at Greensboro), CHARLES J. WYSOCKI, LINDA M. WYSOCKI (Monell Chemical Senses Center, Philadelphia, PA) and MAŁGORZATA KRUCZEK (Jagellonian University, Krakow, Poland and Monell Chemical Senses Center)*

Reproduction in female prairie voles can be activated by contact with male voles or exposure to the male's chemical cues. In these experiments, the vomeronasal organ was removed from females who then underwent an exposure paradigm that limited the amount of contact with males but allowed access to their chemical cues. Sham-operated females (SHAM) also were tested. Females were paired with males for 12 hours and then spent 40 additional hours in the soiled cages of the males before undergoing a behavioral test for activation followed by confirmation by determining ovarian and uterine weights. In the first experiment, 2/3 of the nulliparous SHAM females, but none of the VNX females, exhibited lordosis; SHAM females also had greater organ weights than did the nulliparous VNX females. Hence, at least for females undergoing their initial activation of reproduction, information in chemical cues from the male appears to be processed by the vomeronasal system which subsequently initiates changes in neuroendocrine status. We repeated the experiment, but with one important change; primiparous females, experienced in all phases of the reproductive effort, provided the data. We will report on the effects of experience prior to VNX on subsequent male-induced activation of reproduction.

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Contribution of Acidic Compounds to Gender Specific Scent Images in the Tamarin, Saguinus fuscicollis. A.M. BELCHER, G. EPPLE and AMOS B. SMITH, III (Monell Chemical Senses Center and Dept. of Chemistry, Univ. of PA, Phila., PA).

In the saddle-back tamarin, a South American primate, chemical signals play an important role in socio-sexual communication. Specialized skin glands in the circumgenital-perianal area produce secretions which are deposited, together with some urine and genital discharge, as complex scent marks. These scent marks contain a large number of volatile, as well as high molecular weight components, which together produce the chemical messages. Scent marks deposited by both males and females contain a variety of information relating to the reproductive and social condition of the individual. One of the messages contained in the scent relates to gender. The monkeys spontaneously discriminate, in a two-choice test, between scent from males and scent from either females or from castrated males. Thus, it appears that hormone-dependent constituents in scent from males contributes to this discrimination. To investigate the role of acidic, basic and neutral compounds in providing hormone-dependent cues for maleness, basic-neutral and acidic extracts of aqueous pools of scent from males, females and castrated males were prepared. These extracts were tested in a behavioral bioassay and analyzed chemically by GC/MS. Results of the behavioral assays indicate that the acidic, but not the basic-neutral fraction, retains cues for maleness. Analysis by GC/MS documents that the acidic extract from males contains compounds which are not present in the corresponding basic-neutral fraction, or in either the basic-neutral or acidic fractions from females or from castrated males. Identification of these compounds and their significance for the construction of cues for gender discrimination is currently under investigation, using both behavioral and chemical analytical techniques.

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Rat Olfactory Bulb has High Levels of Glycogen as Measured by *In Situ* Freezing. ROBERT COOPERSMITH, SUZANNE M. COOPER and MICHAEL LEON (Dept. of Psychobiology, University of California, Irvine CA 92717)

We have previously shown histochemically that rat olfactory bulb has high levels of glycogen phosphorylase, indicating high glycogen turnover. We have also shown rapid glycogen turnover in olfactory bulb *in vitro* slices, which is regulated by olfactory bulb neurotransmitters and modulators. We now report that olfactory bulb has a high steady-state level of glycogen compared to other brain areas, *in vivo*. To measure olfactory bulb glycogen, which is rapidly broken down after death, Wistar rats ranging in age from 0 day (day of birth) to 20 months were briefly anesthetized, the bulbs exposed and rapidly frozen *in situ* with isopentane at -80°C, followed by removal with cold instruments to avoid thawing. The same method was used to obtain samples from neocortex (frontal pole). Tissue samples were then assayed for glycogen by amyloglucosidase digestion followed by spectrophotometric determination of glucose. Endogenous glucose and glucose-6-phosphate in the tissue were controlled for. At all ages, bulb had at least twice the glycogen level as cortex. During the first week of life, bulb glycogen was extremely high; on day 0 the level was 20.8 μmol/gm wet wt, falling to 12.4 μmol/gm by day 3, and reaching the adult value of approximately 5 μmol/gm by day 14. The adult cortical level was approximately 2 μmol/gm. We also have preliminary data which indicates that bulb glycogen may be responsive to odor stimulation. Four-day old rat pups exposed to peppermint odor for 10 min immediately before bulb freezing had 30-40% higher glycogen levels than clean air-exposed controls. We are currently further investigating this aspect of bulb glycogen metabolism.

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Evidence of Functional Topography Following Complete and Partial Bulbectomy

Kathleen M. Guthrie, J. M. Holmes and M. Leon (Dept. of Psychobiology, University of California, Irvine CA 92717.

Functional topography in the olfactory epithelium projection to the rat main olfactory bulb can be demonstrated with ^{14}C 2-deoxyglucose (2-DG). Uptake of 2-DG in focal regions of the glomerular layer in response to odor stimulation reflects olfactory nerve activity and may have functional significance for olfactory bulb coding. Studies have shown that destruction of olfactory bulb areas containing these foci does not impair olfaction. However, there is a possibility that the highly plastic components of the system reorganize sufficiently by the time of behavioral testing to establish a new functional topography in the remaining bulb tissue. Indeed, anatomical tracing studies using WGA-HRP have shown that the olfactory epithelium attempts to remap in its entirety onto olfactory bulb fragments (Shipley et al., 1987).

We addressed the following questions: 1.) Can functional topography be demonstrated in bulb fragments? and 2.) Does the olfactory nerve demonstrate functional activity when in contact with a novel target? We performed unilateral complete or partial bulbectomies on male Wistar rats at postnatal day (PN) 5-9 (birth = PN0). Three to ten weeks later, rats were injected with ^{14}C 2-DG prior to odor exposure, and the brains processed for autoradiography. We observed regions of focal 2-DG uptake, both in bulb fragments and in regions of the forebrain containing ectopic glomerular structures. These results provide evidence of functional topography within lesioned bulbs and additionally suggest that the olfactory nerve is capable of functional activity when in contact with the forebrain.

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Neurobehavioral correlates of olfactory preference and aversive associative conditioning in infant rats. REGINA M. SULLIVAN* and DONALD A. WILSON (Developmental Psychobiology Laboratory, Department of Psychology, University of Oklahoma).

The preweanling rat olfactory bulb demonstrates a modified response to odors which have acquired positive value through classical conditioning. The present study examined 1) whether the bulb is similarly modified by aversive conditioning and 2) whether these learned neurobehavioral responses can be extinguished.

Wistar pups received daily conditioning sessions from PN1-PN17. Experimental pups received daily 10 min, forward pairings of ODOR & STROKING (tactile stimulation) or ODOR & SHOCK. Control pups received daily random presentations of ODOR & STROKING or ODOR & SHOCK, or were left undisturbed. On PN18-PN19, half of the pups from each training condition underwent extinction which consisted of 10 min exposures to odor alone, 4 times/day. On PN20, pups were tested in one of 3 tests: behavioral odor preference, olfactory bulb focal 2-DG uptake, or mitral/tufted cell single-unit response patterns to the conditioned odor. The results suggest that: 1) both olfactory aversion and preference conditioning modifies olfactory bulb responses, 2) no differences can be detected between modified bulb responses to learned aversive odors and to learned preferred odors, and 3) both aversion and preference conditioned neurobehavioral responses can be readily extinguished.

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Time course of olfactory deprivation-induced changes in olfactory bulb function. DONALD A. WILSON* (Developmental Psychobiology Laboratory, Department of Psychology, University of Oklahoma).

Olfactory deprivation during postnatal development induces profound changes in olfactory bulb neuroanatomy and neurochemistry (Meisami, 1976; Baker, 1986; Frazier & Brunjes, 1988). It has recently been demonstrated that this deprivation similarly modifies olfactory bulb function (Wilson, Guthrie & Leon, submitted; Guthrie, Wilson & Leon, submitted). Deprivation from PN2-PN20 enhances focal 2-DG uptake and mitral/tufted cell responsiveness to odors and enhances presumed granule cell inhibition of mitral/tufted cells. The present study examined the time course of deprivation effects on bulb physiology.

Rat pups were cold anesthetized at PN1 and had a single naris cauterized. Control pups were cauterized on the top of the snout. On PN10, PN20 or PN40, rats were anesthetized with urethane and evoked responses to single and paired shocks of either the lateral olfactory tract (LOT) or olfactory nerve (ON) were examined. The results suggest that paired-pulse inhibition induced by stimulation of the LOT is enhanced in deprived bulbs. This enhanced inhibition is not significant until 20 days of deprivation and increases by PN40. On the other hand, preliminary results suggest that inhibition induced by paired-pulse stimulation of the ON is not detectably modified by deprivation at any of the ages tested here.

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Can Rats Smell If They Have One Olfactory Bulb Removed and the Contralateral Naris Closed?

NANCY L. HUNT, ALEJANDRA J. PAZOS & BURTON M. SLOTNICK (The American University)

In Experiment I, six adult rats trained on an amyl acetate detection task were unilaterally bulbectomized and tested with the naris ipsilateral to the intact bulb sewn shut. These rats had little or no loss in detection of 0.5-0.005% (of vapor saturation) amyl acetate.

In Experiment II, one naris was closed by cauterization in six 1-6 day old rats. When adult, these rats were trained to detect 0.5-0.005% amyl acetate and to discriminate between linalyl acetate and ethyl acetate. They were then retested on these tasks in 100-trial sessions after removal of the bulb ipsilateral to the open naris. Relative to their pre-operative performance, these rats had a slight decrease only in initial (first 20 trials) performance on the 0.005% amyl acetate detection task and the 2-odor discrimination. Bilaterally bulbectomized rats performed at chance as did unilaterally bulbectomized rats tested without differential vapor cues.

These results demonstrate that vapors inhaled through one nostril can stimulate olfactory neurons in the contralateral nasal vault. We assume that this inter-nasal communication is mediated by the nasopharyngeal canal (an opening in the ventral posterior nasal septum of the rat) and/or by vapor refluxed from the mouth via the nasopharynx. Thus, in this study and in prior anatomical studies on odor deprivation, unilateral naris closure may have only partly deprived ipsilateral receptor neurons of vapor stimulation.

Quantification of the Effects of Unilateral Naris Closure on the Olfactory Bulb of Adult Mice. JEFFREY R. HENEGAR and JOEL A. MARUNIAK (Division of Biological Sciences, University of Missouri-Columbia, Columbia MO).

Naris closure for a month or more in adult mice causes significant shrinkage of the ipsilateral olfactory bulb. The underlying morphological changes in the olfactory bulbs were assessed using quantitative histological techniques. Four sections from each animal, equally spaced along the rostro-caudal axis, were stained with cresyl violet and analyzed for mitral cell number, area of the external plexiform layer(epl), area of the granule cell layer(gcl), and for granule cell number. Mitral cells were counted using a light microscope and a comparison of the number on the open versus closed side was made. No significant difference in mitral cell number was found. The areas of the epl and the gcl were measured using a computer image analysis system. The area of the closed-side epl was significantly smaller than the open-side epl(41% smaller). The open-side epl areas were compared to control epl areas and no significant difference was found. The area of the closed-side gcl was significantly smaller than open-side gcl(25% smaller). When the open-side gcl areas were compared to the control gcl areas no significant difference was found. Granule cell counts are in progress. It appears that there is a decrease in granule cell number in the closed-side olfactory bulb since that gcl showed shrinkage. Since the mitral cell number did not decrease, granule cell loss could explain the shrinkage of the epl.

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Deafferented Main Olfactory Bulb Glomeruli Have Elevated Levels of Glial Fibrillary Acidic Protein. MICHAEL POSTON, MOLLY BAILEY, RICHARD AKESON and MICHAEL SHIPLEY (University of Cincinnati).

Following injury of the mammalian olfactory epithelium (OE), the axons of newly generated primary olfactory neurons (PONs) extend into the deafferented main olfactory bulb (MOB), form synapses on second order neurons and provide some functional recovery. We have shown previously (Neurosci. Abstr. 15: 976, 1989) that, at timed intervals following two different lesion paradigms, the level of glial fibrillary acidic protein (GFAP) expression in the glomeruli of deafferented MOBs is much greater than in control MOBs. Transport from the OE to the deafferented MOB of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) suggested that the deafferented glomeruli had the greatest increases in GFAP expression. However, this could not be confirmed since the methodologies for the immunohistochemical localizations of GFAP and WGA-HRP were incompatible and separate animals had to be used. Here, we have instead employed unconjugated WGA to label olfactory axons, using antibodies to WGA to visualize its transport to the MOB. By comparing adjacent sections from the same bulb we can directly confirm that deafferented glomeruli with little or no WGA staining exhibit the highest levels of GFAP expression. Thus, removal of PON input results in a gliotic response by the MOB glomerular astrocytes, including increased GFAP levels and hypertrophied processes. Studies to elucidate the duration of this GFAP scar and the relationship of regenerating PON axons with reactive glomerular astrocytes are now in progress.

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Transplant of Fetal Brain Tissue into the Olfactory Bulb of Adult Rats. JOHN H. McLEAN AND QUOC TRAN. *(Memorial University of Newfoundland, St. John's, Nfld., Canada A1B 3V6).*

In Alzheimer's disease (AD), several regions of the brain degenerate, including the olfactory bulb. Three specific regions having widespread projections to cortices are often depleted in AD resulting in loss of cholinergic, serotonergic, and noradrenergic inputs to the cortex. This loss presumably contributes to memory, arousal, and mood impairments that are indicative of AD. One means of providing replenishment of depleted pathways in the brain is to provide the brain with trophic and/or neural transplants. In these initial experiments, we have transplanted cholinergic, serotonergic and noradrenergic neurons from fetal (E15-17) diagonal band, raphe and locus coeruleus, respectively, into olfactory bulbs that have been previously depleted of centrifugal afferent inputs. The contralateral olfactory bulb serves as control for influence of afferent inputs/trophic substances on transplant survival. Following survival of 2 to 8 weeks, the animals were sacrificed by perfusion and the olfactory bulbs were cut frozen at 30 μm. The sections were analyzed by immunocytochemistry and AchE histochemistry to determine the location and degree of integration of transplanted cells into the host brain. Cholinergic and serotonergic neurons generally exhibited good survival and integration into the host olfactory bulb while noradrenergic cells did not survive well. Serotonergic axons from transplants appeared to grow particularly well and preferentially innervated glomeruli, a major target of these axons in the normal bulb. We are continuing the analysis to determine if the transplanted cells appear to connect with the host brain in a normal manner.

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ANATOMICAL EVIDENCE FOR ALTERATIONS IN RECEPTIVE FIELDS OF ROSTRAL NST NEURONS DURING NORMAL POSTNATAL DEVELOPMENT. PHILLIP S. LASITER (Florida Atlantic University).

Normal postnatal development of the rostral gustatory NST in rat involves a progressive caudal expansion of the chorda tympani and greater superficial petrosal terminal field (CT/GSP). The present study evaluated whether the expansion of the CT/GSP terminal field envelops a greater number of neurons that project axons to the parabrachial nucleus (PBN). Fluorescent dual labeling experiments were conducted whereby all CT/GSP axons were labeled with lucifer yellow (LY), and all PBN projection neurons in the NST were labeled with propidium iodide (PI). Analyses revealed several novel findings. First, the caudally-directed expansion of CT/GSP terminal fields envelops a greater number of PBN projection neurons in NST. Second, the majority of PBN projection neurons in the NST are located caudal to the CT/GSP terminal field and rostral to the glossopharyngeal terminal field; During normal development CT/GSP axons migrate to regions of the NST that contain the highest density of PBN projection neurons. Third, during development a greater number of PBN projection neurons are labeled in the NST, and the majority of these cells are located caudal to the CT/GSP terminal field and rostral to the glossopharyngeal field. Finally, immunohistochemical studies using antibodies directed toward LY and PI show that during early development large multipolar and fusiform cells are predominately labeled, whereas during late postnatal development small multipolar and fusiform cells are predominately labeled. Results show that (1) the CT/GSP terminal field envelops additional PBN projection neurons during normal development, (2) "path finding" in CT/GSP axons is directed toward the region of the NST that contains the greatest density of PBN projection neurons, (3) a large proportion of axons destined for PBN targets presumably migrate to the PBN during late postnatal development, and (4) morphological heterogeneity within the gustatory NST of adult rats is at least partially related to somatic characteristics of neurons that establish connections with the PBN during either early or late development.

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Pre- and postnatal development of the rostral nucleus tractus solitarius (NTS) and geniculate afference in hamster. DIANE L. KACHELE, MARK C. WHITEHEAD (Ohio State University) and PHILLIP S. LASITER (Florida Atlantic University).

Fluorescent nerve-labeling methods were used to investigate the development of the geniculate afferent (GA) terminal field in hamsters aged embryonic day 10 (E10) through adulthood. Changes in the density and somatic area of NTS neurons in the GA terminal field also were investigated during ontogeny. Labeled GA axons enter the rhombencephalon at E10. At E11, although the NTS has not differentiated, labeled axons course through the marginal layer of the primitive medulla. During E12, the NTS becomes recognizable histologically and labeled GA axons descend in the solitary tract, sending a few terminal fibers from lateral to medial into the rostral NTS. At E13-E15, the NTS is well delineated, outlined medially and rostrally by a border that contains few neurons and laterally by the solitary tract. More labeled GA axons enter the NTS, which is characterized by general densicellularity as at all prenatal ages. At birth, labeled axons form a denser terminal field and individual terminal fibers bear numerous small swellings as they course medially. Neuronal density in the GA terminal field at birth is two-thirds that in the E12 hamster and density decreases further over postnatal age. Neuronal density in the NTS at postnatal days 10-15 (P10-P15) is one-tenth and density in the adult NTS is one-twelfth that at E12. Terminal field volume of GA axons increases four-fold from P1 to adultlike volumes at P20-P25. Decrements in neuronal density during ontogeny at least partly result from decreases in the number of neurons from E12 until P10-P15, because the number of neurons in the GA terminal field of the neonate is twice that in the P10-P15 hamster. Density also likely decreases as somatic area increases, inasmuch as mean neuronal area triples between E12 and P10-P15. Increases in terminal field volume of GA afferent axons during ontogeny may occur paripassu with primary afferent axonal growth and branching, increases in neuronal area and dendritic growth of surviving neurons in the GA terminal field. These results document dramatic morphogenetic changes in the rostral NTS and its GA axons during pre-and postnatal development. Supported by NIH DC00452.

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Restriction of Dietary Sodium During Early Development Alters the Salt Responses of NST Taste Neurons: Reduced Responses in "Deprived" Rats, Hyper-responsivity in "Recovered" Rats. **MARK B. VOGT & DAVID L. HILL (University of Virginia).**

Alterations in normal afferent activity during certain periods of development can have enduring effects on the neurophysiology of central sensory structures. In the taste system, whole chorda tympani nerve (CT) responses to sodium salt stimuli are reduced in rats reared on a low sodium diet from early gestation. Responses to non-sodium salts are unaffected. These effects are not permanent, however, because normal CT salt responses "recover" when deprived rats are fed a sodium replete diet. The CNS neurons that receive taste input from the fibers of the CT are located in the rostral portion of the nucleus of the solitary tract (NST). To learn how changes in afferent salt responses arising from early dietary sodium restriction influence the neurophysiological activity of NST neurons, we recorded taste responses in DEPRIVED rats (N = 18 neurons) reared on a low sodium (0.03%) diet from embryonic day 3 to adulthood, rats RECOVERED (N = 40 neurons) from early deprivation that were fed a sodium replete diet for at least 3 weeks before recording, and NORMAL rats (N = 35 neurons) always fed a sodium replete diet. For sodium salt stimuli (0.05, 0.10, 0.50M NaCl and Na Acetate; 0.10M $NaNO_3$), response frequencies of NST neurons were significantly reduced in DEPRIVED rats compared to NORMAL rats. However, responses in RECOVERED rats were greater than in NORMAL rats. Responses to non-sodium salts (0.05, 0.10, 0.50M NH_4Cl; 0.10M $CaCl_2$ and KCl) did not differ for DEPRIVED, NORMAL and RECOVERED rats, although for the most effective stimuli there was some trend for responses in RECOVERED rats to be greater than those in NORMAL rats. In addition, NST recordings were made in CONTROL DEPRIVED rats (N = 25 neurons) reared to adulthood on a sodium replete diet and then placed on the low sodium diet for at least 4 weeks prior to recording. Responses to sodium and non-sodium salt stimuli did not differ for CONTROL DEPRIVED and NORMAL rats, indicating that the effects of the low sodium diet are restricted to early development. These neurophysiological alterations associated with early sodium restriction may relate to neuroanatomical changes we report at this meeting.

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Restriction of Dietary Sodium During Early Development Alters the Central Anatomical Organization of the NTS. **CAMILLE TESSITORE KING & DAVID L. HILL (University of Virginia)**

Dietary sodium deprivation instituted early in prenatal development produces physiological, anatomical, and functional changes in the developing gustatory system. Neurophysiological recordings from the chorda tympani nerve (CT) demonstrate that whole nerve responses to NaCl are reduced in deprived rats while responses to non-salt stimuli are unaffected. Furthermore, a rearrangement of the CT terminal fields within the nucleus of the solitary tract (NTS) is observed in deprived rats, while glossopharyngeal (IX) terminal fields remain similar to controls. Peripheral responses to NaCl "recover" to control levels when deprived rats are fed a NaCl replete diet; however, the altered pattern of CT innervation within the NTS remains. In addition, the size of the "recovered" CT terminal field is 3X the size of control and deprived fields.

The morphology of the cells in the rostral pole of the NTS is also affected by NaCl deprivation. Large multipolar cells and fusiform cells (projection neurons) show increases in dendritic length and/or number, while there is no effect of deprivation on ovoid cells (interneurons). In "recovered" rats, the projection neurons remain deprived-like; however, ovoid cells show dramatic increases in either the number or length of their dendrites.

Since the NTS volume in deprived rats is 84% that of controls while that of the "recovered" rats is intermediate in size, we are currently investigating the possibility that changes in cell density might contribute to the morphological changes we have observed. It is possible that unique cellular interactions develop within the "classic" gustatory NTS and/or with cells in more medial/caudal divisions of the nucleus. Consequently, alterations in electrolyte and fluid homeostatic mechanisms may be affected, in addition to the documented changes in central taste function and taste-related behaviors.

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Neuronal Geometry during Development of a Functionally Defined Region of the Nucleus of the Solitary Tract. CHARLOTTE M. MISTRETTA (Schools of Dentistry and Nursing, University of Michigan, Ann Arbor, MI 48109), MARY WOMBLE and SUAT GURKAN (University of Michigan)

Between fetal and perinatal periods in sheep there is a marked increase in convergence of taste papilla input onto second order taste neurons in the nucleus of the solitary tract (NST). The period of convergence is accompanied by altering response frequencies to salt stimuli. To learn whether there are differences in neuron geometry that relate to development of convergence, we are studying Golgi-Cox preparations of NST cells from fetuses at 110 and 130 days of gestation (term = 147 days), perinatal animals at about one week before or after birth, and postnatal lambs aged 30 to 50 days. In all except 110 day fetuses, neurons are reconstructed in horizontal sections that include a region of the NST previously marked with an electrolytic lesion. The lesion is made in neurophysiological experiments to locate cells with receptive fields on the extreme tongue tip. Preliminary analyses demonstrate that there are multiple cell types present at all ages. However, there is an apparent increase in proportion of elongate neurons relative to multipolar or stellate types, in lambs compared to younger groups. Average number of dendritic end points does not alter with development, although neurons with very high branch orders (seventh and eighth) are sometimes seen in perinatal animals and lambs. Soma size is larger in perinatal animals than in any other group. Using total length spanned by dendrites in the medial-lateral divided by rostral-caudal direction, an orientation ratio of 0.73 is found in 130 day fetuses and this increases progressively to 1.49 in lambs. This developmental reorientation would align dendrites in parallel with afferent taste fibers. The existence of relatively complex neuron geometries in young fetuses could have a permissive role in establishment of multiple, convergent innervation. Although basic cell types are present in the youngest fetuses, our data also suggest some developmental remodeling in NST neurons.

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Electrophysiological Studies of the Pectinal Chemosensory System of the Scorpion. D.D. GAFFIN and P.H. BROWNELL (Oregon State University, Corvallis, OR 97331-2914)

Behavioral studies of scorpions indicate the pectinal sensory appendages function as general chemoreceptors for substrate borne chemical stimuli. In male sand scorpions (Hadrurus, Paruroctonus) the frequency of pectine tapping of the substrate is especially vigorous in areas contaminated by conspecific females or by extracts of their cuticle. Structurally, the pectines support an ordered array of minute, peg-shaped sensilla, each with a slit-like terminal pore innervated by 10-18 sensory neurons. In recent studies we used metal and glass electrodes to record from the tips and bases of individual peg sensilla and characterized their responses to stimuli of various modalities. Mechanical deflection of a sensillum triggered firing of a single, fast-adapting unit, while water contact with the terminal pore stimulated strong responses from at least two other receptor cells. Primary alcohols, aldehydes, ketones, and esters (C5-C9) and carboxylic acids (C1-C4) gave dose dependent responses when applied directly to the sensillum or in puffs of air blown over the pectine. Responses to pure substances which differ only in functional group or carbon chain length were distinguishable by peak firing frequency of one or a few cells and by latency of response onset. For example, one class of receptor cells was exited by hexanol while hexanal inhibited these units and stimulated a second receptor type. Responses to esters and ketones were biphasic with an initial period of inhibition followed by sustained excitation. Thus, by electrophysiological criteria, the pectines of scorpions appear to be chemoreceptors of general sensitivity. In a behavioral context they appear to fill the same role as the antennal chemosensory systems of other arthropods.

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Uptake of Immunoglobulins by Olfactory Receptor Neurons. THOMAS A. BAKER and JOEL MARUNIAK (Division of Biological Sciences, University of Missouri, Columbia MO).

Olfactory receptor neurons (ORNs) are known to take up and provide a route into the CNS for a variety of substances and pathogens. IgG, IgA and IgE are found in human nasal mucus. Uptake by ORNs and subsequent transport to the CNS might provide a route past the blood brain barrier, either for the body's own immunoglobulins, or experimentally, for antibodies conjugated to various compounds. We nasally irrigated adult mice with solutions of either murine or non-murine immunoglobulins to find out if mouse ORNs take up their own or non-murine immunoglobulins, and whether they are transported into the CNS. The presence or absence of the immunoglobulins in cells of the olfactory and respiratory epithelia and in the CNS was determined immunohistochemically. Olfactory receptor neurons were found to take up a variety of immunoglobulins. The fate of these immunoglobulins and their transport into the CNS will also be discussed.

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Trace Metal Content of Olfactory Bulbs in Alzheimer's and Parkinson's Disease. J. EVANS, L. HASTINGS (Dept. of Environmental Health, Univ. of Cincinnati), L. OLSON and B. SHEPPARD (Dept. of Chemistry, Univ. of Cincinnati).

Degenerative brain diseases such as Alzheimer's and Parkinson's Diseases are often associated with pathological and functional changes in the olfactory system. The fact that the olfactory system can serve as a route of entry for toxic compounds into the central nervous system has led some to suggest that a relationship may exist between exposure to certain toxicants and degenerative brain disease. To examine this relationship, human olfactory bulb tissue from deceased subjects diagnosed with Alzheimer's or Parkinson's Disease as well as controls was analyzed to determine the levels of a variety of elements. Multi-elemental analysis was performed using inductively coupled argon plasma interfaced to a quadruple mass spectrometer. Frozen tissue samples (5 control, 5 Parkinson's and 10 Alzheimer's) were weighed, transferred to clean quartz test tubes, digested in 1 ml HNO_3 and diluted to 10 ml in distilled deionized water. Data analysis revealed comparible levels of the trace metals Zn, Cu and Fe as well as Rb and Sr across the the three sample groups. However, Br levels were significantly higher than controls in the Alzheimer's samples ($p<.05$), but not in the Parkinson's samples. No significant difference was observed between the three groups for the toxic metals of Pb, Hg, Sn, Cd, Ni, Cr and Mn, but levels of Al were significantly elevated over controls in both of the degenerative disease sample groups ($p<.05$). Although the significance of the findings with bromide are not known, the results support a possible relationship between increased toxic metal levels and degenerative disease for at least one metal, aluminum. The nature of this relationship remains to be determined.

This work was supported by NIEHS grant ES04099 and an NIEHS Supplemental Instrumentation Award.

Effects of Unilateral Naris Closure on the Rate of Neurogenesis in the Olfactory Epithelium of Adult Mice. FRANK COROTTO and JOEL MARUNIAK (Division of Biological Sciences, University of Missouri, Columbia MO).

In adult mice, surgical closure of one external naris results in detrimental effects to the open side of the nose. The specific effects depend on the duration closure. Two to three months of closure causes extensive loss of receptor cells on the open side. After four months there is a complete recovery of the receptor cell population. Longer periods of closure again result in extensive loss of receptor cells. We are investigating possible causes of this apparently cyclic population phenomenon by examining rates of neurogenesis in the olfactory epithelium following unilateral naris closure. We use bromodeoxyuridine to label cells which are synthesizing DNA and immunohistochemical methods to detect the label. We will report on: the presence or absence of a caudo-rostral gradient in the rate of neurogenesis in control animals, and the effects of naris closure on the rate of neurogenesis in the rostral, middle and caudal olfactory epithelium of the open and closed sides in animals closed for 1, 2, 3 and 4 months. Implications of the results on models of receptor cell population dynamics will be discussed.

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Complete Dependence of Maturation of Olfactory Receptor Neurons in the Postsuckling Rats on Thyroid Hormones. MARK PATERNOSTRO and ESSIE MEISAMI (Dept. of Physiology, Univ. of Illinois, Urbana, IL 61801).

We have previously shown that the surface area of rat olfactory epithelium (OE) and total number of olfactory receptor neurons (ORNs) increases 20 fold from birth to 90 d. Animals made hypothyroid from birth by the addition of propylthiouracil (PTU) (0.1% w/v) to the drinking water, showed a 40% reduction in OE growth during the suckling period; in postsuckling hypothyroid rats, there was no more increase in surface area of OE or ORN number. Animals allowed to recover from the hypothyroid retardation at weaning (25 d) by withdrawal of PTU, showed a marked recovery in OE growth, resulting in complete compensation of OE surface area and ORN number by 90d. To examine the role of thyroid hormones on maturation of ORNs, we determined the surface density of ORN dendritic knobs in (1u) sections obtained from nasal septum of normal and hypothyroid animals at various ages during the postnatal period. Dendritic knobs are characteristic of mature ORNs (1 knob/mature ORN). The surface density of the knobs (no/ sq mm of OE) increases significantly during the postnatal period, reaching a value of 60,500 in 90d rats. Assuming this value to be 100%, the density was found to be 45% and 70% in newborn and 25d normal rats respectively. By the age of 25d, hypothyroid pups were found to have about a 25%($p<0.05$) reduction in this parameter compared to 25d normal animals, indicating a delay or retardation in maturation of ORNs. After weaning, no significant increase in the surface density of knobs was observed in the hypothyroid rats, so that by 90d the deficiency had increased to 45%. The recovering rats showed marked increase in densities compared to hypothyroid rats. Work on the extent of the recovery is in progress.

Supp.: NIH Training Grant & Univ. Ill. Res. Board.

Development of the Olfactory Epithelium: A Combined Histochemical and 2-Deoxyglucose Study in the Mouse. DAVID S. REASNER & ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 USA)[1]

Environmental information received *in utero* either directly or indirectly through maternal means can provide obvious adaptive advantages to mammals that rely on an immediate behavioral response to their surroundings after birth. In particular, there is increasing physiological and anatomical evidence that the capacity to detect and store chemosensory information before birth exists in at least some rodents. Chemical signals detected prenatally could be used postnatally to recognize the dam, other kin, or the nest and possibly, dietary factors in the local population. Our studies of this problem in mice, originally centered on the accessory olfactory system (AOS) because seminal 2-Deoxyglucose (2-DG) studies with the rat demonstrated enhanced labelling of the accessory olfactory bulb *in utero*. We subsequently determined in the mouse that the canal connecting the AOS to the nasal cavity is not patent before birth (Coppola & O'Connell, *Neuroscience Letters* 105:680-688, 1989) and that foci of metabolic activity revealed with the 2-DG technique are present prenatally in the main but not the accessory olfactory epithelium (Coppola & O'Connell, *Chemical Senses* 14:691 1989). Thus in the mouse, the AOS is unlikely to play a role in prenatal olfaction and we have, therefore, begun to concentrate on the main olfactory epithelium (OE). The foci of metabolic activity observed in our preliminary comparison of the main and accessory OE may reflect the energetic demands of either sensory or developmental events. Therefore, we sought to examine the distribution of cell proliferation in the main OE. We have now combined a histochemical marker of cell proliferation (bromodeoxyuridine incorporation) with the 2-DG technique in order to compare the pattern of this developmental activity with the overall distribution of metabolic activity in the main OE of the fetal mouse. Cell proliferation in the OE is heavy but remarkably patchy and although labeling shows some topographical distribution, the pattern of cell proliferation does not appear to specify the concurrent metabolic activity. Future studies will compare these distribution patterns following chemosensory manipulation of the prenatal environment.

[1] We thank Michael Attella for his help on this project. Supported by NIDCD grants DC00131 & DC00371.

Regional odor stimulation of glandular activity in the olfactory epithelium.

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S E Dyson University of Western Australia

Odor stimulation of the rat nose produces distinct regions of high metabolic activity in the Bowman's gland layer of the olfactory epithelium. No comparable deep activity is found in respiratory epithelium. The radioactive 2-deoxyglucose method was combined with histological methods for identifying mucopolysaccharides. Areas of low metabolic activity were found to be associated with absence of depletion of mucus contained by goblet cells in respiratory epithelium. Presence of mucopolysaccharides in the mucus layer of the olfactory epithelium was not correlated with deep metabolic activity, suggesting that odour-driven secretions are dispersed well before the end of the 45 minute experimental period required by the 2-DG technique. Other histological techniques are being investigated to provide information on the relationship between odour reception and localized secretory zones. This study suggests that secretions may contain substances with odorant-specific qualities, possibly to assist reception or to consume the odorant in a manner akin to a post-synaptic neuro-transmitter enzyme.

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Expression of Carbohydrate Antigens on Rat Olfactory Neurons. G.A. SCHWARTING (E.K. Shriver Center), M. YAMAMOTO and J.E. CRANDALL (E.K. Shriver Center), Waltham, MA 02254.

We have previously described the expression of three unique carbohydrate antigens (CC1, CC2, and 1B2) in the rat olfactory system. Antibodies to CC1 react with an N-acetylgalactosamine containing glycolipid and is expressed in the vomeronasal organ (VNO) or vomeronasal nerve (VNN) and in the rostral half of the accessory olfactory bulb (AOB). CC2 antibody, which reacts with α-galactose and α-fucose-containing glycolipids and glycoproteins is expressed in the VNO, VNN and AOB and also on a subset of the main olfactory epithelium (OE), olfactory nerve (ON) and in dorso-medial glomeruli of the olfactory bulb (OB). 1B2 antibodies, which react with a β-galactose terminal glycolipid, is expressed on the luminal surface of cells in the VNO and a subset of OE cells. Cells taken from embryonic day 15 rat olfactory epithelium are grown in serum free medium on a laminin substratum and are maintained for up to 2 weeks. Within a few days of plating, the laminin surface is coated with a layer of non-neuronal cells. Olfactory epithelial neurons grow on the surface of the non-neuronal monolayer either in large groups or as individual cells. Immuno- fluorescence studies have demonstrated the presence of CC1, CC2 and 1B2 positive cells in culture.

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Human Odor Intensity Perception: Correlation with Frog Epithelial Adenylate Cyclase Activity and Transepithelial Voltage Response. DEBORAH S. KREISS, RICHARD L. DOTY, RICHARD E. FRYE (Smell and Taste Center, David Mahoney Institute of Neurological Sciences, and Department of Otorhinolaryngology and Human Communication, School of Medicine, University of Pennsylvania, Philadelphia, PA)*

Although a number of odorants depolarize frog olfactory receptor cells by binding to ciliary glycoproteins which activate membrane-bound G proteins to induce adenylate cyclase-mediated increases in intracellular cAMP (cyclic adenosine 3',5'-monophosphate), it is not known whether these odorants influence human odor perception via similar mechanisms. In this paper we present evidence derived from odor attribute ranking and multidimensional scaling procedures that the perceived intensity of such odorants to humans is correlated with (a) the amount of adenylate cyclase activity they induce in an in vitro frog olfactory cilia preparation and (b) the magnitude of their influence on the frog transepithelial voltage response or electro-olfactogram (EOG). These observations lend support to the hypotheses that (a) the perception of odor intensity by humans is associated with cAMP-related epithelial processes and (b) the decreased olfactory sensitivity observed in G_s-deficient pseudohypoparathoid patients may arise from G_s protein deficiencies at the receptor level. These findings imply that remarkable homologies exist between the intensity-related olfactory receptor processes of frog and man, in accord with the notion of conservation of gene sequencing for receptors among diverse vertebrate forms.

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Pertussis toxin substrates and G-protein-like immunoreactivity in the olfactory organ and CNS of the spiny lobster. TIMOTHY S. MCCLINTOCK (Yale University School of Medicine), SAMUEL C. EDWARDS (University of South Florida), BARRY W. ACHE (The Whitney Laboratory).

Proteins potentially belonging to the family of GTP-binding proteins were identified by ADP-ribosylation catalyzed by pertussis toxin and by immunoblot reactions with polyclonal antisera raised to peptides from mammalian G-proteins (Mumby et al., 1986, Proc. Natl. Acad. Sci., USA 83:265). Pertussis toxin ribosylated identical patterns of protein bands in membrane preparations from aesthetasc hairs (containing olfactory dendrites) and antennular lumen (containing olfactory receptor cell somata) - major doublets at 39 and 37 kDa and three minor bands of lower apparent mass, visualized on SDS-PAGE gels. In comparison, only a doublet at 38 kDa was ribosylated in brain. Preliminary immunoblots revealed that antibody U49 (common β-subunit peptide) labelled bands of 32 kDa in the antennular lumen and 33 kDa in brain. Antibody J881 (common Gα peptide) strongly labelled bands of 38 kDa in the antennular lumen and of 36 kDa in brain. In contrast, antibodies raised to mammalian peptide sequences specific to $G\alpha_s$, $G\alpha_o$ and transducin failed to strongly label bands in the expected 30 - 50 kDa range.

These results indicate that the olfactory organ expresses G-proteins different from those found in the lobster's CNS. We hope to further test the hypothesis that one or more of these proteins are G-proteins involved in olfactory transduction.

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Specificity of Olfactory Receptor Neurons for Pheromones and Host Odors in the Boll Weevil, Anthonomus grandis Boh. (Coleoptera:Curculionidae). JOSEPH G. DICKENS (U. S. Department of Agriculture, Agricultural Research Service, Boll Weevil Research Unit, Mississippi State, MS 39762)

Detection and coding of chemical signals in a complex chemical environment by insects and other animals are dependent on the sensitivity and specificity of the olfactory receptor neurons involved. Orientation to and discrimination of chemical signals emitted by its host plant and mates is of particular importance to the boll weevil, since it feeds and develops only on the cotton plant, Gossypium hirsutum, or other closely related Malvaceae. Electrophysiological investigations of single receptor neurons on the antennae of boll weevils revealed 11 types of receptor neurons as characterised by their responsiveness to pheromone components and selected host plant odors. Four types of receptor neurons were responsive to four components (I, II, III, and IV) of the insects' aggregation pheromone. Neurons responsive to I discriminated between its two optical isomers. II neurons responded to II with lesser though significant responses to III. One neuron responded exclusively to II with no significant response to III. IV neurons responded exclusively to this pheromone component. Seven types of receptor neurons could be classified based on their responsiveness to the following individual plant odors: benzaldehyde; trans-2-hexen-1-ol and other six carbon alcohols and aldehydes (i. e. green leaf odors); the monoterpenes, trans-β-ocimene and linalool; and the sesquiterpenes, β-caryophyllene, β-bisabolol, and gossonerol. Receptor neurons for plant odors generally responded only to a specific plant odor or closely related analogs, and were as responsive as, or in some instances more responsive than, receptor neurons for pheromones at the same stimulus load. The specificity of the individual receptor neurons is discussed with regard to chemical signals emitted by the insects' host plant and conspecifics.

Initial Characterization of Inositol-1,4,5-trisphosphate Binding to Isolated Olfactory Cilia. D. LYNN KALINOSKI, ARDITHANNE G. BOYLE, SCOTT ALDINGER and DIEGO RESTREPO (Monell Chemical Senses Center, Philadelphia, PA)

Previous experiments from this laboratory have demonstrated that stimulus amino acids elicit G-protein-linked stimulation of phosphoinositide turnover resulting in the elevation of intracellular inositol-1,4,5-trisphosphate (IP_3) in olfactory cilia from the channel catfish (Ictalurus punctatus). In an accompanying abstract (Teeter and Restrepo) we report the identification of a calcium channel in the plasma membrane of olfactory cilia which is directly gated by IP_3. Studies of the specific binding of IP_3 to membranes closely associated with the endoplasmic reticulum have been used to characterize a related channel which mediates the release of calcium from internal stores (Ferris et Al, Nature 342:87-89, 1989 and Spät et Al, Nature 319:514-516, 1986, Worley et Al, JBC 262:12132-36, 1987). In studies with [^{3}H]-IP_3, we find that IP_3 binding to olfactory cilia is specific and saturable with a K_d of 1.3±0.3 uM and an estimated number of binding sites of 72±25 pmol/mg protein (mean±SEM, n=3). Specific binding has a pH-optimum at physiological pH and is relatively insensitive to the concentration of calcium in the medium (from <1 nM to 1 mM). In these respects, this binding site displays different binding properties from those previously reported for IP_3 binding to microsomes. The biochemically determined binding affinity is consistent with the affinity for activation of the IP_3-gated calcium channel detected following incorporation of catfish cilia plasma membrane vesicles onto phospholipid bilayers.

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Development of the Olfactory Epithelium in Normal and Hypothyroid Xenopus Larvae. GAIL D. BURD and LAURIE THOMAS (University of Arizona)

We previously reported that cell genesis is accelerated in hyperthyroid Xenopus larvae and that within 10 days this results in a 2 fold increase in the number of olfactory axons in the olfactory nerve (Burd, 1988. Soc. Neurosci. Abstr.; Burd, 1989. In Olfaction and Taste X. In Press). Propylthiouracil (PTU) blocks the synthesis of thyroid hormone; we showed that treatment with PTU reduced by 50% the number of axons in the olfactory nerve. Here we present additional data supporting the hypothesis that thyroid hormone is important for development of olfactory receptor cells. Larvae were bred and raised in our laboratory until stage 48 and were then randomly placed into individual tanks containing a dilute salt solution (controls) or a salt solution with 0.01% PTU. After 8 days when the larvae were stage 50, a subpopulation from the control and experimental groups received 1 uCi of ^{3}H-thymidine I.P. After 24 hours, the animals were anesthesized and fixed, and the tissue was embedded in Durcupan and processed for autoradiography. The remaining animals were processed in the same manner when the control animals reached stage 58. Stage 50 (before the normal rise in thyroxine levels) and stage 58 (after thyroxine levels have started to rise and before metamorphic climax) were selected to compare the effects of normal levels of thyroid hormone on cell genesis and epithelial thickness of the olfactory epithelium. Our results showed that PTU treatment had no effect on the thickness of the olfactory epithelium at stage 50, but prevented the increase in thickness of the olfactory epithelium normally observed at stage 58. PTU did not block cell genesis in the olfactory epithelium at either stage, but there may be a reduction in the number of new cells generated in the PTU treatment group at stage 58. We hypothesize that thyroid hormone may be necessary for normal cell genesis and neuronal maturation in the developing larval olfactory epithelium. Supported by the Whitehall Foundation.

Development of Calcitonin Gene-Related Peptide in the Mouse Olfactory System. HARRIET BAKER (Cornell University Medical College).

Recent studies demonstrated that olfactory receptor neurons exert trophic influences over the development and maintenance of phenotype in the olfactory bulb (OB). The nature of the trophic control is not known. Denis-Donini (1989) utilized dispersed cultures of OB and epithelium to investigate trophic interactions. These data suggested that calcitonin gene-related peptide (CGRP) was localized to olfactory receptor cells and regulated the expression of the dopamine phenotype in OB. Although prior studies indicated that CGRP was not present in adult rodent receptor neurons, the possibility existed that receptor cells transiently express CGRP during development. The studies reported here utilized immunocytochemical techniques to determine the in vivo distribution of CGRP during development of the mouse OB. The antiserum (provided by S. Amara and R. Blakely) was the same as that utilized in the dispersed cultures. Development of receptor neurons was monitored with antiserum to olfactory marker protein (OMP, provided by F. Margolis). OMP was demonstrable in olfactory receptor cells and their processes in gestational day 13 (E13) embryos. CGRP was not found in receptor cells or their processes at E13 or any other gestational age. However, from E13, CGRP-containing cells and processes were found in spinal cord among other CNS areas. By E15, varicose processes, presumably of trigeminal origin, existed in the lamina propria but not among the receptor neurons. The number and intensity of staining of these processes increased in later gestational ages and some of the fibers appeared to innervate the OB. These studies demonstrate that in vivo, in contrast to observations in vitro, CGRP cannot be demonstrated in olfactory receptor cells during embryogenesis.

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Olfactory Neurogenesis: genetic or environmental controls? ALBERT I. FARBMAN & VIRGINIA McM. CARR (Dept. of Neurobiology, Northwestern University, Evanston, IL 60208)

Neurons in the vertebrate olfactory and vomeronasal organs are unique in that, under physiological conditions, they are continually replaced throughout the life of the animal. In mammals, it has been estimated that olfactory neurons have a life span of approximately a month, in amphibians, longer. It is commonly believed that basal cells in the olfactory epithelium undergo mitoses, that daughter cells of mitoses differentiate and mature into functional sensory cells as they make synaptic connections with the bulb, and they subsequently die and are removed.

Experiments in the past decade have shown that, under certain conditions, olfactory neurons can survive for as long as 12 months in mice. These data suggested that in the absence of disease-related destruction of the olfactory epithelium, most olfactory cell death occurs among newly formed or not fully mature cells that fail to establish synapses with the olfactory bulb. In other words, the life span may be determined by extrinsic factors, related to nutrition, disease, age, hormonal state, injury, etc. In any cell population, it can be expected that the life span of given individuals, if represented on a Gaussian curve, would have extremes. Some olfactory cells can be long-lived, and we have recent evidence that, in control animals, a small number is very short-lived. The number of short-lived cells is increased following bulbectomy. It is therefore possible to perturb the olfactory cell population in various ways to increase or decrease the mean life span and up-regulate or down-regulate the rate of mitosis.

Thus, there remains little doubt that environmental influences can govern survival time of olfactory neurons. However, there is ample evidence to support the notion that the life span of the sensory cell is genetically programmed. The answer to the question posed in the title, then, is that both genetic and environmental influences regulate olfactory neurogenesis.

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Incorporation of ^{3}H-thymidine in the Embryonic Vomeronasal and Olfactory Epithelia of Garter Snakes. DAVID HOLTZMAN and MIMI HALPERN (State University of New York, Health Science Center at Brooklyn).

Previous studies have shown that the vomeronasal (VNE) and olfactory (OE) epithelia of adult vertebrates provide good models for studying normal neuronal turnover and regeneration in response to axotomy. However, little is known about the cell dynamics in the embryonic VNE and OE or the origins of different cell types in these structures. Using ^{3}H-thymidine autoradiography, both in vivo and in vitro, the origins of receptor and supporting cells and the survival of labelled cells in the embryonic VNE and OE of garter snakes were examined. The results of this study suggest that the receptor and supporting cells of both epithelia arise from separate stem cells and that two subpopulations of stem cells exist for receptor cells in the embryonic VNE. One subpopulation generates cells that migrate through the receptor cell columns, while another subpopulation remains at the base of the epithelium for approximately 50 days. Although it is unclear how long receptor cells in the embryonic OE survive, the results of this study suggest they survive at least 37 days and may survive over 56 days. The results of this study suggest that development in these sensory epithelia is different in early versus late embryonic ages and that regeneration in the VNE and OE in adult garter snakes is similar to development during late gestation. Cells in the receptor cell layer of the OE lose their ability to incorporate ^{3}H-thymidine before those in the VNE, suggesting that the onset of neuronal maturation occurs earlier in the OE than in the VNE.

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Neonatal Learning Increases a Focal Olfactory Bulb Neuronal Population. CYNTHIA C. WOO (University of California, Irvine) and MICHAEL LEON (University of California, Irvine)

Young rat pups can be conditioned to approach an odor following odor exposure that is briefly paired with tactile stimulation for the first 18 postnatal days. Associated with this behavioral preference is increased uptake of 14-C 2-deoxyglucose (2-DG) in focal regions of the glomerular layer in the olfactory bulb (Coopersmith and Leon, 1984). To determine whether any changes occurred in the underlying glomerular layer neuronal population of these regions of elevated 2-DG uptake, neural size and number were examined in conditioned and control pups.

A 19% increase in neuronal number was found to be associated with regions of high 2-DG uptake in conditioned pups compared to controls. There were also increases in both glomerular layer area and periglomerular region area in the conditioned pups compared to controls, similar to that previously reported (Woo et al, 1987). No change in cell density or cell size was observed between groups. Qualitative and quantitative analyses revealed that the overwhelming portion of the increase in neuronal number was due to an increase in periglomerular cells. The increase in these inhibitory interneurons could underlie the suppression of the signals of the output neurons from this region of the olfactory bulb in response to a conditioned odor (Wilson and Leon, 1988).

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Multicenter vs. Single Center Clinical Trials. M.A FOULKES. (National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD)

When the number of collaborating centers in a controlled clinical trial increases beyond a single center, there are numerous ramifications. The design and complexity of conducting the trial, and the inferences from the trial are all influenced. The rationale for a multicenter as opposed to a single center trial are more rapid recruitment of necessary subjects, a potentially more representative sample, and expanded collaboration with investigators with similar interests. The effect of multiple centers on the organizational structure of the trial, communication between participants, quality control, performance monitoring, and data collection within the trial will be discussed. Multiple centers also transform analyses, both interim and final analyses, adding issues such as detecting outlier clinics and treatment-by-clinic interactions that do not exist in a single center trial.

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Functional Recovery of Sodium Responses in Sodium Deprived Rats: Induction by Anesthetics. ROBERT E. STEWART and DAVID L. HILL (University of Virginia)

Rats deprived pre- and postnatally of sodium demonstrate profoundly reduced chorda tympani nerve taste responses specific to sodium salts. When these animals are permitted to ingest as little as 30 ml of isotonic saline, a functional recovery of sodium taste responses occurs. Recovered sodium responses are identical to sodium responses seen in normal animals, and recovery appears to depend upon systemic consequences of sodium absorbtion, rather than upon direct stimulation of receptor elements by sodium. In addition, this recovered response can be completely eliminated by the epithelial transport blocker, amiloride. Recently, we have observed that certain anesthetic agents also induce recovery of the amiloride-sensitive sodium taste response. Pentobarbital and urethane induce rapid (within 2 hours) and robust recovery of sodium taste responses. In contrast, chloral hydrate induces recovery only gradually over a 4 to 6 hour period, if at all. These results, in conjuction with other findings, strongly suggest that some humoral factor(s) may be active in inducing the formation of amiloride-sensitive channels. Our current studies are designed to explore the role of new protein synthesis in the recovery of sodium taste responses.

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Sensory Coding of Deterrent Phytochemicals by Gustatory Organs of the Tobacco Hornworm. FRANK HANSON (University of Maryland Baltimore County) and STEPHEN PETERSON (University of Maryland Baltimore County).

Caterpillars reject many non-host plant species because of deterrent phytochemicals. Rejection is thought to be mediated by stimulation of "deterrent cells", inhibition of "acceptance cells", or more complex codes. This study involves the first of these.

Caterpillars have five taste sensilla on each side, two of which are of primary importance in food plant discrimination. In the tobacco hornworm, one of these, the lateral styloconicum, has been shown to contain a deterrent cell highly sensitive to a variety of antifeedants including caffein, aristolochic acid, and salicin. However, behavioral experiments show that this sensory organ has no measurable effect on feeding on the unacceptable plant, Canna generalis, the common canna lilly. Instead, it is the medial styloconica that is responsible for deterring feeding on this plant. The deterrent cells in this organ have not been previously characterized.

Various solvent extracts of Canna were obtained and tested behaviorally; non-polar extracts stimulated feeding, were highly deterrent. Electrophysiological studies showed that deterrent extracts of this plant elicit a vigorous response from predominantly one cell. Dose-response studies using this extract show that activity in this cell correlates well with behavioral rejection of the extract in the disc test. This suggests that rapid activity in this single cell is all that is needed to code for rejection of this non-host plant.

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Bilateral Lesions of the Chorda Tympani or Glossopharyngeal Nerve Do Not Alter NaCl Preferences in the WKY and SHR. BRADLEY K. FORMAKER and DAVID L. HILL (University of Virginia)

In two-bottle preference-aversion tests, the spontaneously hypertensive rat (SHR) prefers higher concentrations of NaCl than the normotensive Wistar-Kyoto (WKY). It has been suggested that this increased NaCl preference was due, in part, to a decreased sensitivity of the chorda tympani to NaCl. However, we have reported that electrophysiological responses of the chorda tympani were equivalent between the SHR and WKY. In order to examine further the role of the peripheral gustatory system in mediating the NaCl preference of these two strains, we studied two-bottle choice preferences in three groups of rats: 1) sham operated, 2) bilateral chorda tympani lesioned and 3) bilateral glossopharyngeal lesioned. Twenty-four hour, two-bottle preference tests were conducted every other day between distilled water and one of four NaCl concentrations: 0.05, 0.25, 0.154 and 0.5M. Each concentration was presented once and the position of the bottles was alternated each day. On days when preference tests were not conducted, two bottles of distilled water were made available. NaCl preference was defined as the ratio NaCl intake to total fluid intake in a 24 hour period. Preliminary results indicated that lesioned animals were equivalent to shams in their preference for NaCl. That is, the SHR continued to ingest and prefer higher concentrations of NaCl than the WKY in all groups studied. Thus, bilateral denervation of either the anterior or posterior tongue was ineffective in altering the strong behavioral preference and aversion for NaCl in the SHR and WKY, respectively. The effect of a combined bilateral chorda tympani and glossopharyngeal lesion on the 24 hour two-bottle NaCl preference is currently being investigated in these strains.

Quantitative Development of Taste Buds in the Human Fetus. RUOYU XIAO and INGLIS MILLER, JR., (Dept. Neurobiology & Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC)

Several studies have described the morphological development of taste buds in the human fetus, but less information is available on taste bud proliferation. Tissue samples were obtained from fetal tongues at autopsy. The gestational ages were from about 3 to 6 months as judged by fetal length and menstrual history. Tissues were fixed with paraformaldhyde and picric acid, and embedded in paraffin. Serial sections were cut, mounted on slides and stained with H & E. Taste buds on fungiform, foliate and vallate papillae were followed through serial sections, and each taste bud was counted only once. The locations of taste pores,if present,were identified. Preliminary observations follow:

Gestational Age (months)	N (fetuses)	Fungiform (Avg. tot. tb/half tongue with pores)	Foliate	Vallate
3	4	138.5	1.0	27.5
	range	(47-220)	(0-3)	(14-40)
6	2	358	352	221
	range	(317-399)	(192-512)	(128-315)

Fungiform taste buds seem to develop earlier than vallate and foliate taste buds. Over 90% of these gustatory fungifrom papillae have only one taste bud, but multiple taste buds are usual in the adult tongue.

We thank the Dept. of Histol. and Embryol., West China Univ. of Med. Sci., Chengdu, PRC for providing tissue from their human fetal specimens. Supported by NIH Grant NS 20101.

Distribution of Substance P-Immunoreactive Nerve Terminals in Gustatory Regions of the Hamster Solitary Nucleus. HEATHER J. DUNCAN, SHERYL K. BRINING, and DAVID V. SMITH (University of Cincinnati College of Medicine)

Immunoreactivity to substance P (SP-I) is distributed peripherally in fibers associated with taste buds (Finger, 1986; Nishimoto et al., 1982) and in cells of the petrosal and nodose ganglia (Helke & Hill, 1988). Studies of the central distribution of SP-I afferent fibers show significant projections into the nucleus of the solitary tract (NST). These investigations in the rat delineate the SP distribution in the caudal NST much more carefully than in its more rostral portions because of their focus on cardiovascular and respiratory function (Kalia et al., 1981; Kawano & Chiba, 1984; South & Ritter, 1986). In order to relate the distribution of SP to the gustatory portions of the NST, we compared SP-I to the distribution of HRP-labeled afferent terminals of the hamster's glossopharyngeal (IXth) nerve and to published accounts of its chorda tympani (CT) nerve projections (Whitehead & Frank, 1983). In some animals, HRP crystals were placed into the IXth nerve in the vicinity of the petrosal ganglion. After a 2-day survival, the brainstem was cut at 40 *um* and processed for HRP histochemistry using TMB as the chromagen. In the immunohistochemical experiments, hamsters were perfused with 4% paraformaldehyde and the brains were cut at 30 *um*. Sections were incubated with anti-SP (Incstar) at 1:2000 dilution, placed in secondary antisera, reacted with avidin and biotin (ABC Elite), and SP-I was visualized with DAB. SP-I was distributed throughout the NST and within the spinal trigeminal complex and the dorsal motor nucleus of X, being quite extensive in the caudal NST at the level of the area postrema, as previously described in the rat. There was overlap between SP-I and the IXth nerve projection within the more rostral portions of NST. At the level of the caudal extent of the dorsal cochlear nucleus, SP-I was distributed within the region of afferent termination of the CT nerve. This relationship between SP-I and taste afferent projections suggests a role for SP in gustatory processing.

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An Examination of the Projection from the Gustatory Cortex to the NTS in the Hamster. J.A. LONDON, C.B. HALSELL. M.B. BARRY, T.S. DONTA (Dept. BioStructure and Function, Center for Neuroscience, UConn Health Center)

While several studies have shown the insular cortex projects to the nucleus tractus solitarius (NTS) we have begun to examine this descending projection in detail, with particular attention being paid to the gustatory NTS and gustatory cortex. Glass microelectrodes recorded multiunit activity in the rostral NTS in response to application of a search stimulus (0.03 M NaCl, 0.1 M KCl and 0.1 M sucrose) to the anterior tongue. The boundaries of this taste active region were delimited. A glass microelectrode filled with 2% wheat germ agglutinin horseradish peroxidase (WGA-HRP) was then inserted into the center of the taste active region, taste activity was confirmed, and WGA-HRP iontophoretically injected. With large injections, retrogradely filled neurons were found in the 5th layer of the ipsilateral and contralateral agranular insular cortex. The heaviest label was located in the contralateral cortex. Retrogradely filled cells were located in the granular insular cortex as well as agranular insular cortex. Retrogradely filled neurons were found ipsilaterally in the following areas: central nucleus of the amygdala, lateral hypothalamus and the medial frontal cortex. There was a small amount of label in the contralateral lateral hypothalamus. Injections of WGA-HRP were also made into the insular cortex covering areas previously identified as containing neurons responsive to search solution stimulation of the anterior tongue. Anterograde label was found bilaterally throughout the rostro-caudal NTS. The contralateral label was always heavier. The lateral, central and medial subdivisions of the NTS had relatively equal amounts of label. Anterograde label was also seen in the medullary reticular formation just ventro-lateral to the NTS. This study has shown the location of specific forebrain neurons which directly project to a gustatory region in the NTS. Knowing the origins of these descending neurons allows a better understanding of their role in sensory perception.

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Specificity of Visceral and Pharyngeal Taste Reflex Systems in the Goldfish, *Carassius auratus*. L. E. GOEHLER, B. BOTTGER, & T. E. FINGER. Department of Cellular & Structural Biology, Univ. Colorado Health Sci. Ctr., Denver, CO 80262.

In the goldfish, *Carassius auratus*, the vagus nerve innervates coelomic viscera and a pharyngeal chewing organ which contains taste buds. Afferent nerve fibers from the viscera and those from the pharyngeal chewing organ terminate in separate subnuclei of the general visceral nucleus of the caudal medulla (Morita & Finger, *J. Comp. Neurol.*, 1987). Visceral afferents project to the medial subnucleus (GVm) whereas pharyngeal afferents project to the lateral subnucleus (GVl). These subnuclei may serve as the first synaptic relays for separate reflex systems regulating the digestive system and chewing organ.

In order to assess the anatomical specificity of potential reflex systems regulating digestive and chewing functions, the anatomical tracers horseradish peroxidase (HRP) or the carbocyanine dye DiI, were injected into either the GVm or GVl. Survival times of HRP-injected fish were 7-21 days. DiI was used in paraformaldehyde-fixed brains and incubated at room temperature for 21 days.

Injections of the tracers into GVm resulted in labeled fibers terminating mostly in the dorsal motor nucleus of the vagus, which contains preganglionic parasympathetic visceromotor neurons. In contrast, injections into GVl labeled fibers terminating in the vagal lateral motor column, which contains motor neurons innervating the pharynx. Thus, the neural connections of GVm and GVl seem selective, and appropriate to the tissues innervated by the afferent input to GVm and GVl. These results suggest that general visceral functions are represented by specific organotopic reflex systems in the caudal medulla.

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In vitro electrophysiology from a primary gustatory nucleus: the vagal lobe of goldfish. T.E. FINGER (Dept. C. & S. Biology) & T.V. DUNWIDDIE (Dept. of Pharmacology, Univ. Colorado Health Sci. Ctr., Denver, CO 80262).

The vagal lobe of goldfish is a laminated evagination of the mid-medulla which contains the reflex circuitry required to carry out intraoral food sorting (Finger; *Brain, Behav. Evol. 1988*). Primary gustatory sensory fibers course through the deep fiber layer to terminate in more superficial layers; motoneurons innervating the orobranchial apparatus lie in the deeper layers. In an attempt to define the neurotransmitters involved in transmission of vagal gustatory information, a vagal slice preparation has been developed for *in vitro* electrophysiology. For these experiments, the vagal lobe is removed from cold-anaesthetized goldfish, sliced on a vibratome at 500 μm and placed in artificial CSF. The tissue slices then are permitted to recover for at least 30 minutes in a slice recording chamber prior to recording. A bipolar stimulating electrode is used to stimulate small fascicles in the primary afferent fiber layer; a glass pipette recording electrode is positioned under visual control at different loci in the vagal lobe. A bimodal population response with negative peaks at 3 and 5 msec following stimulation can be evoked at restricted locations in the vagal lobe. This negative-going response is most prominent in layers 6-8 -- the principal layers of termination of the primary sensory fibers. The maximal response is recordable only for a distance of 0.5 - 0.7 mm across the surface of the lobe confirming anatomical results showing that each fascicle of primary afferent fibers ramifies only within a limited expanse of the lobe. These responses are attributable to synaptic currents since they disappear upon removal of calcium from the bathing solution. Further, the later response is more labile to changes in stimulus characteristics and calcium removal and thus may be due to higher order synaptic activation of intrinsic neurons of the vagal lobe. Preliminary results utilizing the glutaminergic antagonist DNQX indicate that this later negative peak may be due to activation of a glutaminergic system.

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Behavioral effects of descending input from the gustatory neocortex to the parabrachial pons in the rat. S. MONROE & P.M. DI LORENZO (Dept. of Psychology, SUNY at Binghamton, Binghamton, N.Y. 13901)

Although it is known that the gustatory neocortex (GN) sends direct projections to the parabrachial nucleus of the pons (PbN), the function of this descending input in the neural processing of gustatory information remains a mystery. The present experiments were designed to investigate the effects of disruption of the corticofugal input to the PbN on taste-related behaviors. Because the PbN projects bilaterally to the thalamus (which in turn projects to the GN) but receives primarily ipsilateral descending input from the GN, it is possible to disrupt much of the GN-PbN input with unilateral lesions in the PbN and contralateral GN. Three experiments were conducted on separate groups of animals with these asymmetrical lesions. The first experiment consisted of a 24 hr, two bottle taste preference test. Stimuli were three concentrations of each of five test stimuli: NaCl, HCl, quinineHCl, sucrose and Na-saccharin. Both consumption of and preference for the highest concentration of NaCl (.2 M) were significantly enhanced in experimental animals compared with controls. In the second experiment, consumption of taste stimuli was measured in 15 min, one bottle tests where all animals were deprived of fluid for 23.75 hrs. This short period of exposure presumably minimized the postingestional effects of the stimuli on the amount consumed. The same stimuli that were used in the first experiment served as test stimuli. Enhanced preference for .2 M NaCl was also observed in experimental animals in the short term tests. In addition there was a significantly lower preference for the lowest concentrations of sucrose (.1 M) and Na-saccharin (.2 %) in experimental animals compared with controls. In the third experiment, three daily 15 min presentations of .1% Na-saccharin were paired with administration of LiCl (.3 M, 1% b.w., i.p.) in a standard conditioned taste aversion paradigm. Experimetnal animals showed learning curves that were indistinguishable from those of control animals. Collectively, the results of these experiments suggest that corticofugal input to the PbN may play some role in the determination of the amount of intake for some tastants but does not affect the potential to form conditioned taste aversions.

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Lesions of the Thalamic Gustatory Nucleus Produce Deficits in Taste Sensitivity and in Cue Salience.
H. K. RENTMEISTER, S. SHEELAR, K. MARTIN, AND B.M. SLOTNICK (The American University)

Rats trained using operant conditioning to detect delivery of 0.005 ml of 1% NaCl received lesions of the thalamic gustatory nucleus and were retested. Unoperated, sham-lesioned controls and those with lesions outside of the target area had virtually perfect retention of the detection task. However, those with lesions of or just posterior to the thalamic gustatory nucleus performed at chance in 1000 postoperative trials (200 trials/daily session). These rats were able to re-acquire the detection task when tested with 5% or 10% NaCl and, in subsequent sessions, most achieved criterion performance of 85% correct when tested again with the 1% NaCl stimulus. When tested with lower concentrations, control rats were able to detect 0.0125% NaCl; most experimental rats were able to detect 0.5% NaCl but performed at chance on a 0.1% test concentration. For all test concentrations experimental rats made many more errors than did controls in achieving criterion performance. These results indicate that lesions of the thalamic gustatory nucleus produce two separate deficits. First, there is a decrease in taste sensitivity. Second, there is a decrease in the salience of the taste cue. This latter deficit is evident from the fact that experimental rats demonstrated detection of moderate concentrations (1% and 0.5%) of NaCl only after they were trained on relatively high concentrations (10% and 5%) of the tastant.

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Intensity Coding in the Gustatory Cortex of the Alert Cynomolgus Monkey. CARLOS R. PLATA-SALAMAN, VIRGINIA L. SMITH AND THOMAS R. SCOTT (University of Delaware).

Gustatory evoked activity of 62 single neurons in the primary taste cortex of two alert cynomolgus monkeys was analyzed to determine neural response thresholds and intensity-response functions. Stimuli were distilled water, fruit juice and several concentrations of the four prototypical taste stimuli: 10^{-4}M to 1.0M glucose; 10^{-4}M to 1.0M NaCl; 10^{-4}M to $3x10^{-2}$M HCl and 10^{-5}M to $3x10^{-3}$M quinine HCl. The response criterion was a stimulus-induced change in activity $\geq$ 2.12 s.d. ($p<.01$) from spontaneous rate, sustained for 5 sec. Mean spontaneous activity was 3.0±3.9 spikes/sec (range=0.0-15.1 spikes/sec). The mean breadth of tuning coefficient was 0.65±0.21 (range=0.00-0.98). By our criterion, neural response thresholds corresponded to stimulus concentrations of 10^{-1}M glucose, 10^{-2}M NaCl, 10^{-3}M HCl and 10^{-4}M quinine, all similar to psychophysical thresholds determined in humans. Mean response rate increased with concentration in an orderly manner for all tastants except HCl, which was also the least effective stimulus. A taste space was generated based on the similarity of response profiles evoked by each pair of stimuli. Subthreshold concentrations of each stimulus formed a group near water; suprathreshold concentrations of glucose and NaCl were situated in separate, coherent clusters, while those of HCl and quinine were intermixed. Thus, stimuli that taste sweet or salty to humans appear to maintain a distinctly consistent quality across concentrations, while it may be more difficult to distinguish between chemicals that are sour or bitter.

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Virginia L. Smith

Quality Coding in the Gustatory Cortex of the Alert Cynomolgus Monkey. VIRGINIA L. SMITH, THOMAS R. SCOTT AND CARLOS R. PLATA-SALAMAN (University of Delaware).

Gustatory evoked activity of 53 single neurons in the primary taste cortex of two alert cynomolgus monkeys was analyzed to study coding characteristics across a range of taste qualities. Stimuli included those that humans describe as sweet (sucrose, glucose, fructose, aspartame), salty (NaCl, LiCl) sour (HCl, citric acid, acetic acid) and bitter (quinine HCl, $CaCl_2$, $MgCl_2$, KCl, Na_2SO_4) plus MSG and distilled water. Mean spontaneous rate was 3.5±2.9 spikes/sec (range=0.0-10.5 spikes/sec). The mean breadth of tuning coefficient was 0.76±0.18 (range=0.0-0.99). Sweet and salty stimuli were generally more effective than those that were bitter or sour. Correlations between pairs of stimulus profiles confirmed similarities and dissimilarities that were anticipated based on psychophysical data. In a taste space generated from these correlations, sugars and salty salts each formed a distinct and coherent cluster. Bitter and sour stimuli plus water were organized into identifiable groups adjacent to one another. Aspartame lay between the sweet and sour-bitter clusters; MSG was on the salty-salt side of the bitter-tasting group. Thus, relative taste quality as defined by responses in the gustatory cortex of the monkey is largely in accord with that reported by humans.

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Coding of Sweet Stimuli in the Gustatory Cortex of the Alert Cynomolgus Monkey. THOMAS R. SCOTT, CARLOS R. PLATA-SALAMAN and VIRGINIA L. SMITH (University of Delaware).

Gustatory evoked activity of 47 single neurons in primary taste cortex of three alert cynomolgus monkeys was analyzed. Stimuli were distilled water, NaCl, HCl, quinine HCl and 19 chemicals of varying molecular structure that elicit predominantly sweet sensations in psychophysical studies. Mean spontaneous activity was 5.0±2.2 spikes/sec (range=0.0-16.9 spikes/sec). The mean breadth of tuning coefficient was 0.59±0.29 (range=0.00-0.93). A taste space was constructed from the relative similarity of response profiles generated by each stimulus. The sweet chemicals formed a coherent cluster within the space with a mean correlation between each pair of profiles of +0.77 (N=171; range =+0.14 to +0.97). At its central core were the simple carbohydrates: glucose, fructose, sucrose and maltose. Nearest them were sorbitol, Ca cyclamate, aspartame and cranberry-raspberry juice. In the next concentric ring were acesulfame K, xylose, xylitol, sorbose, polycose and myoinositol. On the fringes of the sweet cluster were Na saccharin, stevioside, neohesperidin DHC, tryptophan and monellin. A comparison with psychophysical data[1] measuring relative similarity among many of the same chemicals revealed a remarkable degree of concordance. Thus, neurophysiological responses in the macaque provide generally accurate predictions of human experiences of sweetness.

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[1]Schiffman, S.S., Reilly, D.A. and Clark, T.B. (1979). Qualitative differences among sweeteners. Physiol. Behav. 23:1-9.

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Coding of Sodium and Lithium Salts in the Gustatory Cortex of the Alert Cynomolgus Monkey. CARLOS R. PLATA-SALAMAN, VIRGINIA L. SMITH and THOMAS R. SCOTT (University of Delaware).

Gustatory evoked activity of 38 single neurons in primary taste cortex of the alert cynomolgus monkey was analyzed. Stimuli were distilled water, glucose, HCl, quinine HCl, fruit juice and 17 sodium or lithium salts most of which elicit predominantly salty sensations in psychophysical studies. Mean spontaneous activity was 3.7±3.3 spikes/sec (range=0.1-13.2 spikes/sec). The mean breadth of tuning coefficient was 0.71±0.19 (range=0.15-0.99). A taste space was constructed from the relative similarity of response profiles generated by each stimulus. The Na-Li salts formed a coherent cluster within the space, with a mean correlation coefficient between each pair of profiles of +0.85 (N=136; range=+0.48 to +0.96). Among the other basic stimuli, glucose had the closest relationship to the Na-Li salts (mean r=+0.60; N=17; range=+0.38 to +0.77), while QHCl (mean r=+0.32; N=17), HCl (mean r=+0.22; N=17) and water (mean r=+0.18; N=17) were quite distinct from the salts. Within the 17-salt cluster, we took NaCl to be the prototypical salty stimulus. Those salts most distant from NaCl were Na_2CO_3(r=+0.68) and MSG (r=+0.74); those most similar were NaBr (r=+0.90) and LiBr (r=+0.88). Thus, while the anion can exert a considerable influence on the neural code for Na-Li salts, it is not sufficient to alter the profile to one that resembles a different basic taste. There is a close relationship between the relative positions of salts in our space and those of the same stimuli in a space generated from psychophysical data[1].

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[1]Schiffman, S.S., McElroy, A.E. and Erickson, R.P. (1980). The range of taste quality of sodium salts. Physiol. Behav. 24:217-224.

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Coding of Acids in the Gustatory Cortex of the Alert Cynomolgus Monkey. VIRGINIA L. SMITH, THOMAS R. SCOTT, and CARLOS R. PLATA-SALAMAN (University of Delaware).

Gustatory evoked activity of 51 single neurons in the primary taste cortex of three alert cynomolgus monkeys was analyzed. Stimuli were distilled water, fruit juice, glucose, NaCl, quinine HCl and 21 inorganic and organic acids. Mean spontaneous activity was 3.1±3.6 spikes/sec (range=0.0-17.5 spikes/sec). The mean breadth of tuning coefficient was 0.73±0.16 (range=0.26-0.98). The acids, at a concentration of 10mM, were rather ineffective stimuli, eliciting a mean net response of just 2.1 spikes/sec (vs 2.1 spikes/sec for water, 2.4 spikes/sec for 0.3M NaCl, 3.0 spikes/sec for .001M QHCl, 6.9 spikes/sec for 1.0M glucose, and 9.1 spikes/sec for 20% fruit juice). Nonetheless, there were distinct differences among the individual activity profiles they evoked. Pyruvic acid generated a profile rather similar to those of glucose and quinine; glutamic and tannic acids elicited patterns that were highly correlated to all three non-acid prototypes, but less well with most acids. In general, the acids formed only a diffuse cluster in the taste space, with a mean intercorrelation between each pair of profiles of +0.70 (N=210; range=+0.16 to +0.93). Their profiles were rather closely related to those of water (mean r=+0.69; N=21) and quinine HCl (mean r=+0.62; N=21), and more poorly to the profiles generated by NaCl (mean r=+0.43; N=21), glucose (mean r=+0.41; N=21) and fruit juice (mean r=+0.38; N=21). This is in accord with psychophysical reports that the tastes of a range of acids are predominantly sour, followed in decreasing order by bitter, salty and sweet components.[1]

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Coding of Amino Acids in the Gustatory Cortex of the Alert Cynomolgus Monkey. THOMAS R. SCOTT, VIRGINIA L. SMITH and CARLOS R. PLATA-SALAMAN (University of Delaware).

Gustatory evoked activity of 54 single neurons in the primary taste cortex of two alert cynomolgus monkeys was analyzed. Stimuli were distilled water, glucose, NaCl, HCl, quinine HCl, fruit juice and 16 ℓ-amino acids selected for their wide range of molecular weights and structures, their nutritional significance and for the availability of psychophysical data on their perceived taste qualities. Mean spontaneous rate was 2.4±1.0 spikes/sec (range=0.0-9.5 spikes/sec). The mean breadth of tuning coefficient was 0.71±0.18 (range=0.00-0.99). Amino acids most effective in activating cortical taste neurons were MSG, PRO and GLY. The least effective stimuli were TYR, THR, PHE and TRP. All 22 stimuli were incorporated into a taste space. The clearest distinction was between sweet stimuli (glucose, fruit juice, glycine, proline) and those that were sour-bitter (associated with HCl and quinine) or insipid (allied with water). These findings conform well with electrophysiological results from the peripheral taste nerves of rats[1] and with human psychophysical data[2] for 14 of the 16 amino acids. Only ALA and THR generated neural profiles out of keeping with psychophysically-based predictions, and this result probably stemmed from the rather low concentrations that we used. Thus, the macaque appears to provide an appropriate neural model for human taste perceptions of a range of complex organic molecules.

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Coding of Taste Mixtures in the Gustatory Cortex of the Alert Cynomolgus Monkey. CARLOS R. PLATA-SALAMAN, VIRGINIA L. SMITH and THOMAS R. SCOTT (University of Delaware).

Gustatory evoked activity of 27 single neurons in primary taste cortex of the alert cynomolgus monkey was analyzed in response to stimulation by taste mixtures. Stimuli were distilled water, glucose, NaCl, HCl and quinine HCl, the six dyads of these prototypes, the four triads and the tetrad plus fruit juice. Concentrations were held constant within a mixture: glucose, for example, was presented at 1.0M as the simple prototype, but was mixed in dyads at 2.0M, triads at 3.0M and the tetrad at 4.0M. Mean spontaneous rate was 2.6±3.0 spikes/sec (range=0.0-12.3 spikes/sec). The mean breadth of tuning coefficient was 0.71±0.23 (range=0.00-0.97). Response magnitude to mixtures showed universal suppression. Activity evoked by the six dyads was suppressed by a mean of 46% from the sums of responses to their unmixed components; activity to the four triads was suppressed a mean of 60%; that to the tetrad by 74%. A taste space was constructed to investigate the presumed quality of mixtures. Most mixtures were located between and among their primary components. In particular the tastes of dyads were predictable from the taste qualities of their constituents; this was less so for the triads and the tetrad.

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Glucagon Administration Affects Taste Responsiveness in Rat Nucleus Tractus Solitarius.
BARBARA K. GIZA (University of Delaware), RHONDA O. DEEMS (Sandoz Pharmacy), DENNIS A. VANDERWEELE (Occidental College) and THOMAS R. SCOTT (University of Delaware).

Pancreatic glucagon (PG) administration decreases meal size and increases latency to feed. Hepatic portal vein infusions are most effective, perhaps because they induce glycogenolysis and so stimulate vagal afferents. Chemical factors relating to glucose availability have been shown to influence taste sensitivity in rat nucleus tractus solitarius (NTS). Thus we recorded taste-evoked multiunit activity in the NTS of female rats before and after PG administration to determine whether the resulting satiety is associated with changes in gustatory responsiveness. Taste activity evoked by 0.1M NaCl, 1.0M glucose, .01M HCl and .01M QHCl was recorded through tungsten microelectrodes (Z=300-500 K). If the recording proved stable, 40 ug/kg PG was administered through a hepatic-portal cannula while taste evoked responses continued to be monitored every 90 sec for one hr. Responsiveness to oral application of glucose declined 25% ($p<.05$) during the 20 min immediately following the injection. Evoked responses to NaCl, HCl and QHCl as well as peripheral measures of blood glucose did not differ significantly from pre-injection levels. We hypothesize that a reduced sensitivity to the most appetitive components of a complex taste experience would limit the hedonic appeal that sustains feeding, and so promote manifestations of satiety. Gustatory responsiveness has repeatedly been shown to be sensitive to the condition of the organism. These data provide another instance of this relationship.

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Immunohistochemical Localization of Tyrosine Hydroxylase and Olfactory Marker Protein to the Glomerular Layer of the Human Olfactory Bulb.
CHARLES A. GREER (Yale Univ. Sch. Med.), ROBIN L. SMITH (Yale Univ. Sch. Med.), DENNIS D. SPENCER (Yale Univ. Sch. Med.) and HARRIET BAKER (Cornell Univ. Med. Coll.)

Comparatively little is known about the biochemical organization of the human olfactory bulb (OB). However, due to a growing awareness of the potential importance of the olfactory system for understanding clinical conditions, such as Alzheimer's Disease, it is important that we develop a better comprehension of cytoarchitecture in the human OB. Consequently, we inititated a series of studies utilizing immunohistochemical probes to establish neuronal phenotypes and their laminar segregation. Human OBs were obtained at autopsy, within 8 hrs. post mortem, or during neurosurgical operative procedures. The tissue was fixed for 8 - 18 hrs. in 4% paraformaldehyde in 0.1M phosphate buffer and subsequently processed for immunohistochemical localization of antibodies to olfactory marker protein (OMP) or tyrosine hydroxylase (TH) using Vector Labs ABC *elite* kits. In Nissl stained sections the human OB displayed a laminar organization similar to that observed in most mammals. However, the human OB differed in two ways. First, the laminar boundaries were less rigid. Second, the anterior olfactory nucleus was distributed along the length of the OB deep to the granule cell layer. As previously described by several laboratories, OMP was localized to the most superficial laminae of the human OB. OMP staining appeared throughout the olfactory nerve layer. From this outermost layer, fascicles of axons separated prior to entering the more deeply placed individual glomeruli. Within the glomeruli OMP postive fascicles were interspersed with unstained profiles believed to be the processes of postsynaptic neurons. Both the number of OMP stained glomeruli and the intensity of staining varied between specimens. As described in other species, TH staining was localized to juxtaglomerular neurons which surrounded the glomeruli. The dendritic processes of these neurons, which were also TH positive, extended into the glomeruli. In addition, varicose axons were evident in the interglomerular zones. It was clear that only a subpopulation of juxtaglomerular neurons were positive for TH. As in the case of OMP, the intensity of TH staining varied between glomeruli within as well as between specimens. This is the first demonstration of TH in juxtaglomerular neurons of the human OB. The results from both OMP and TH staining suggest that neuron phenotype and perhaps synapse organization of human OB glomeruli may be comparable to that described for other species.

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Morphological Observations and Distribution of the Human Olfactory Neuroepithelium.
EDWARD E. MORRISON and RICHARD M. COSTANZO (Department of Physiology, Medical College of Virginia, Richmond, Virginia 23298)

The human olfactory neuroepithelium is thought to be a discrete region (2-5cm^2) lining the superior portion of the nasal cavity. In the present study scanning electron microscopy was used to obtain three-dimensional observations of the morphology and distribution of cells within the human nasal cavity. A block of tissue containing the septum and lateral wall of the nasal cavity was obtained at autopsy and placed in fixative. In fractured regions of the epithelium we were able to examine the three dimensional morphology of four different cell types. Olfactory receptor cells, located primarily in the lower two thirds of the epithelium, had dendrites extending to the surface that terminated in ciliated knobs. Olfactory axons formed small fascicles enclosed by a supporting cell sleeve-like process. Supporting cells were columnar, had a microvillar surface, and formed foot like attachments at the basal lamina. Basal cells were irregular in shape and found in the lower epithelial region. Microvillar cells were typically observed near the epithelial surface. They were flask shaped, had a tuft of microvilli and a single axon-like process. By examining a large area of the mucosal surface, we were able to determine the distribution of olfactory regions. Low power observations revealed most of the mucosal surface as respiratory. In the superior region, we observed an irregular distribution of light and dark contrast areas. At higher magnification, these areas corresponded to olfactory and respiratory epithelium. Discrete patches of respiratory epithelium were observed within olfactory areas, even in the most superior regions of the nasal cavity. Our results indicate that the human olfactory neuroepithelium consists of four cell types. We also observed that it is not a uniform sensory area, and that it contains discrete patches of respiratory epithelium. The irregular distribution of the olfactory epithelium points out the potential difficulty in obtaining reliable biopsy samples of olfactory tissue.

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EDWARD E. MORRISON and RICHARD M. COSTANZO (Department of Physiology, Medical College of Virginia, Richmond, Virginia 23298)

The human olfactory neuroepithelium is thought to be a discrete region (2-5cm^2) lining the superior portion of the nasal cavity. In the present study scanning electron microscopy was used to obtain three-dimensional observations of the morphology and distribution of cells within the human nasal cavity. A block of tissue containing the septum and lateral wall of the nasal cavity was obtained at autopsy and placed in fixative. In fractured regions of the epithelium we were able to examine the three dimensional morphology of four different cell types. Olfactory receptor cells, located primarily in the lower two thirds of the epithelium, had dendrites extending to the surface that terminated in ciliated knobs. Olfactory axons formed small fascicles enclosed by a supporting cell sleeve-like process. Supporting cells were columnar, had a microvillar surface, and formed foot like attachments at the basal lamina. Basal cells were irregular in shape and found in the lower epithelial region. Microvillar cells were typically observed near the epithelial surface. They were flask shaped, had a tuft of microvilli and a single axon-like process. By examining a large area of the mucosal surface, we were able to determine the distribution of olfactory regions. Low power observations revealed most of the mucosal surface as respiratory. In the superior region, we observed an irregular distribution of light and dark contrast areas. At higher magnification, these areas corresponded to olfactory and respiratory epithelium. Discrete patches of respiratory epithelium were observed within olfactory areas, even in the most superior regions of the nasal cavity. Our results indicate that the human olfactory neuroepithelium consists of four cell types. We also observed that it is not a uniform sensory area, and that it contains discrete patches of respiratory epithelium. The irregular distribution of the olfactory epithelium points out the potential difficulty in obtaining reliable biopsy samples of olfactory tissue.

Supported by NIH grant DC00165.

Successful Treatment of Phantosmia with Preservation Of Olfaction: A Case Report. DA LEOPOLD, SL YOUNGENTOB, JE SCHWOB, DE HORNUNG, MM MOZELL, HN WRIGHT. (Clinical Olfactory Research Center, SUNY HSC, Syracuse, New York)

Phantosmia is the perception of a smell when there is no stimulus source. We present a young woman who complained of a left sided phantosmia and right brow headaches for eight years. The pain was thought by some to be migrainous, but it responded well to injection therapy. The phantosmia, which was described as a mixture of vinegar and rotten eggs, could be blocked with anesthesia of the left olfactory cleft or occlusion of the left nostril. Although a treatment for unilateral phantosmia had been described which utilized a craniotomy and olfactory bulb ablation, we decided to use a less invasive approach which would disconnect the entire olfactory mucosa from the bulb. An endoscopic intranasal approach was utilized to excise a full thickness sheet of olfactory epithelium from the left cribriform plate area which included all the observable fila olfactoria. Histological evaluation of the tissue excised demonstrated respiratory and olfactory epithelia and olfactory nerve. Two weeks after the procedure both the phantosmia and all olfactory ability were absent on the affected side. Five weeks after the procedure the phantosmia was still absent, however her left nostril olfactory ability had returned to 71% correct on the Odorant Confusion Matrix (OCM). (The best pre-operative score on that side had been 75% correct). The following conclusions may be considered: 1) Since most, if not all, of the fila olfactoria were transected during the surgery, at least some of the primary olfactory neurons were somehow basic to the phantosmia in this patient. 2) Since olfactory ability returned following excision of the fila olfactoria, there is now evidence for the reconstitution of the olfactory nerve in humans. 3) Alternatively, there is always the possibility that a small area of olfactory epithelium with its central connections was not disrupted and that this reduced input was sufficient for olfactory identification as represented by the OCM. Further experience with other patients who have phantosmias will determine if this positive result can be duplicated, and perhaps will elucidate the pathophysiology of phantosmia. Supported by NIH Grant #DC00220

Rhinomanometric Evaluation of Patients with Anosmia. ALFREDO A. JALOWAYSKI (UCSD Medical Center, San Diego, CA) CLAIRE MURPHY (San Diego State University, San Diego, CA and UCSD Medical Center, San Diego) and TERENCE M. DAVIDSON (UCSD Medical Center, San Diego, CA)

Right and left nasal airway resistance (NAR) were measured before and after Neo-synephrine spray in patients with anosmia and in a group of normal controls. Both groups received a battery of tests that included age-adjusted olfactory butanol threshold, odor identification, nasal endoscopy, computed tomographic scan and nasal cytology. Based on the results of these tests, clinical history and physical exam, the patients were divided into diagnostic categories, of which the inflammatory and post-viral contained the largest number of patients.
The table below compares the improvement in NAR after decongestant spray between the three groups of subjects:

	Control	Inflammatory	Post-viral
n	52	60	68
M	5.95	2.04	2.92
S.D.	±10.61	±2.02	±3.63
S.E.	1.37	0.25	0.50

A one-way ANOVA showed the group NAR means to be significantly different, $F(2,177) = 6.07$, $p < 0.003$. A post-hoc comparison shows the inflammatory group to be statistically different from the post-viral group of patients, $p < 0.05$. We conclude that NAR measurement performed before and after decongestant spray is a useful test in characterizing patients with anosmia of different etiology.

Rhythmicity of Nasal Airflow in Children and Adolescents JULIE A. MENNELLA AND GARY K. BEAUCHAMP (Monell Chemical Senses Center)

The 'nasal cycle', evidenced in approximately 60% of normal adults, is characterized by recurring side-to-side alternations in nasal patency. These alternations occur every few hours as a consequence of congestion and decongestion of the vascular epithelium covering the nasal turbinates. Because the study of the nasal cycle has focused almost exclusively on adults, we analyzed nasal airflow changes in 48 children aged 3 to 17 years. Anterior rhinometric observations of nasal airway patency were obtained every ten minutes throughout a 5-hour period. Children younger that 7 years exhibited a pattern different from the reciprocal airflow pattern characteristic of adults. That is, airflow through the two nostrils either changed randomly (38%) or in parallel (44%). Between the ages of 7 and 17 years, however, the adult pattern emerged such that reciprocity of airflow was evidenced in 60% of the subjects tested. Period length was significantly shorter in 7- to 10-year-old children, however, when compared to older children and adults.

Effects of Odorants on Respiratory Behavior and Nasal Patency. DONALD W. WARREN (University of North Carolina at Chapel Hill)

A previously developed technique (Walker et al., 1990; Warren, 1984), which combines psychophysical measurements with continuous recording of nasal patency and respiratory behavior, was used to study the responses of 10 subjects to well-controlled stimulation with acetic acid, amyl acetate and phenylethyl alcohol. In most subjects, nasal patency was reduced by odor exposure, but not by presentation of clean air, and all concentrations caused similar increases in nasal resistance. Changes in respiratory behavior were often seen in the first inspiration after odorant was presented and typically consisted of decreases in air volume per breath. With acetic acid and amyl acetate, increased ratings of Nasal Irritation and decreased tidal volumes were seen as concentration was increased. These responses were not seen with phenylethyl alcohol, although ratings of Odor Strength increased with increases in odorant concentration. These results are consistent with the idea that reduction in tidal volume may serve as a physiological correlate of the perception of irritation. Further, the failure of phenylethyl alcohol to affect either respiratory behavior or the perception of irritation may relate to previous work suggesting that this compound is a relatively poor stimulus for nasal trigeminal chemoreceptors.

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Warren, D. W. (1984) A quantitative technique for assessing nasal airway impairment. Am. J. Orthod. 86, 306-314.
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Chemosensory Function and Appetite in Liver Disease: An Evaluation of 111 Patients. MARK I. FRIEDMAN, RHONDA O. DEEMS, LAWRENCE S. FRIEDMAN, SANTIAGO J. MUNOZ and WILLIS C. MADDREY (Monell Chemical Senses Center and Jefferson Medical College, Philadelphia, PA)

We evaluated chemosensory function, food preferences, and dietary habits in 111 patients with liver disease including those with hepatitis, cirrhosis, primary biliary cirrhosis, sclerosing cholangitis, and hepatic metastatic cancer. Patients were compared to healthy individuals matched for gender and age.

Chemosensory complaints were common in the patients with liver disease; over 40% of patients reported recent taste changes and 27% reported recent changes in ability to smell, compared to only 6% of healthy controls. More patients than controls reported food cravings (47% vs 17%) and food aversions (33% vs 16%). Reported taste changes in patients with liver disease generally indicated decreased acuity; common complaints were that food was "bland" or "tasteless." Despite these chemosensory complaints, no differences were detected in gustatory or olfactory thresholds. Hedonic ratings of food were generally decreased in patients compared to controls. Foods with a predominantly bitter taste were specifically less preferred by patients with liver disease or cancer compared to healthy controls. Hepatitis patients were more likely to report chemosensory disturbances and poor appetite than patients with other types of liver disease.

The findings suggest that disturbances of appetite and changes in food preferences in patients with liver disease are related to the type of liver pathology. These results provide further insight into the role of the liver in the control of food intake and food preferences.

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Chemosensory Dysfunction, Burning Mouth Syndrome (BMS) and Sjogren's Syndrome (SS). APRIL E. SCOTT, M.D., UCONN Health Ctr. Farmington, CT; LESLIE BOUVIER, UCONN Sch. Dent. Medicine.

BMS is described as intraoral burning sensations in the absence of objective findings. SS is a chronic inflammatory disorder characterized by diminished lacrimal and salivary gland secretions. A possible association for BMS with Sjogren's is suggested on review of the medical literature. Historical data from 35 SS, 14 BMS, and 18 control age and sex matched subjects is available. The hypotheses being tested are: (1) that BMS and SS are variants of the same disease process for some patients, and (2) that both BMS and SS groups have chemosensory deficits. Eye dryness was described by 44% of the BMS, 91% of the SS and 8% of the controls, ($p < .05$ for each comparison). Nasal dryness: 69% of the BMS, 91% of the SS group and 43% of the controls, (s vs c, $p=.0002$). Oral dryness was described by 38% of the BMS, 97% of the SS and none of the controls (s vs. c, $p = .002$, rest NS). None of the controls, and 47% of the SS described oral burning. Of those with oral burning, 75% of the BMS and 40% of the SS cited the tongue tip. Onset of burning was sudden in 36% of the BMS and 23% of the SS subjects, occurring spontaneously in 43% of the BMS and 70% of the SS subjects. Dry mouth occurred prior to burning in 24% of the BMS and 72% of the SS subjects. The frequency of history of oral candidiasis, post-menopausal status and estrogen use was not statistically different between the three groups. Although more BMS and SS than controls reported a history of hiatal hernia or esophagitis, this reached statistical significance only for the SS group ($p=.03$). "More sensitive" olfactory ability was reported in 10% of the BMS subjects, 11% of the SS, and none of the controls. For those reporting olfactory loss, 60% of the BMS, 33% of the SS and none of the controls reported fluctuations. Olfactory distortions were reported in 27% of the BMS patients, 20% of the SS, and 8% of the controls. Phantosmias were reported in 25% of the BMS and SS subjects, and 17% of the controls. Dysgeusia was described in 46% of BMS, 45% of SS and 0 controls. Eleven SS subjects have undergone CCCRC testing, 36.4% testing normosmic, 27.3% mildly hyposmic, and 36.4% moderately hyposmic to anosmic. Of the 20 BMS subjects, 50% tested normosmic, 15% mildly hyposmic, and 35% moderately hyposmic to anosmic. Twenty five BMS subjects are in the process of being evaluated for Sjogren's. To date: 2/8 have an elevated ESR, 4/7 a + ANA, 0/6 a + RF, and 0/4 a + ENA. Of 6 lip biopsies, 2 were positive for Sjogren's and the other 4 showed chronic sialoadenitis. All 8 Schirmer's tests were +. Preliminary data show overlap in the clinical characteristics of these two disorders, and documents decreased olfactory function in both groups.

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Taste Performance of Sjogren's Syndrome Patients. J.M. WEIFFENBACH (National Institute of Dental Research, NIH), P.C. FOX (National Institute of Dental Reseach, NIH)

Many Sjogren's Syndrome (SS) patients complain of reduced enjoyment of food and of decreases in their sense of taste. The sensory mechanisms underlying these impairments in the subjective appreciation of taste stimuli are poorly understood. We measured responses of SS patients (n=39) to aqueous solutions representing the four basic tastes. Detection thresholds and direct scaling of intensity by cross-modal matches to distance were obtained. For each quality, patients demonstrated significant differences from controls (n=145 for thresholds, n= 170 for direct scaling) by repeated measures analysis of covariance (covariate =age). Average threshold sensitivity to each quality was significantly reduced in patients. The average slope of individual psychophysical functions was shallower and the consistency of response across presentations was reduced for each quality. In addition to these differences in average performance, patients differed from controls with respect to the frequency and pattern of impaired performance. Impairments, defined as performance below the tenth percentile of age/sex matched controls, were significantly more frequent among patients than controls for each measure. Moreover, among individuals with poor performance for one quality, impaired performance for other qualities was significantly more frequent among patients than among controls for each measure. This dramatically different pattern of taste deficits suggests that the mechanisms producing taste impairment in SS are different from those underlying poor performance by un-affected individuals.

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Drug-related MPTP-Induced Parkinsonism: No Evidence of an Olfactory Deficit. RICHARD L. DOTY (Smell and Taste Center, University of Pennsylvania, Philadelphia, PA), ANU SINGH, JAMES TETRUD, J. WILLIAM LANGSTON (California Parkinson's Foundation, San Jose, CA).

If some forms of parkinsonism are caused by environmental agents which enter the CNS via the primary olfactory neurons and damage the olfactory pathways, then persons whose parkinsonism is the result of intravenously-administered agents would be expected to have normal olfactory function. On the other hand, if the olfactory dysfunction of parkinsonism is secondary to neural processes associated with the motoric dysfunction proper, then such MPTP-induced parkinsonians might be expected to evidence olfactory dysfunction. To address this issue, we evaluated the olfactory function of five of the original seven index cases of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism (Ballard, Tetrud & Langston, Neurology 35:949-956, 1985). All five of these patients evidenced normal olfactory function, as measured by both the University of Pennsylvania Smell Identification Test and a single staircase detection threshold test using the odorant phenyl ethyl alcohol. These preliminary observations suggest that parkinsonism secondary to intravenously administered MPTP differs from most other forms of parkinsonism in not being accompanied by olfactory dysfunction. These data are in accord with the hypothesis that some forms of idiopathic parkinsonism may be caused by agents entering the CNS via the primary olfactory pathways.

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Olfactory System Involvement in the Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex of Guam. DANIEL P. PERL (Mount Sinai Medical Center, New York, NY), RICHARD L. DOTY (University of Pennsylvania, Philadelphia, PA), JOHN C. STEELE (Guam Memorial Hospital, Tamunging, Guam), DANIEL LEVY (Mount Sinai Medical Center), JOHN D. PIERCE, JR. (University of Pennsylvania), KWANG MING CHEN (Guam Memorial Hospital), and LEONARD T. KURLAND (Department of Health Sciences Research, Mayo Clinic, Rochester MN).*

Amyotrophic lateral sclerosis (ALS) and a form of parkinsonism accompanied by dementia occur among the Chamorro natives of Guam in endemic proportions. Although the etiology of this set of disorders remains obscure, detailed epidemiological studies suggest that their high incidence is related to local environmental factors. In this study we demonstrate that Chamorro natives with these diseases evidence significant olfactory dysfunction relative to age- and gender-matched Guamanian controls. Furthermore, we present preliminary evidence that proportionately more asymptomatic controls living in villages associated with the highest incidence of these disorders have abnormal olfactory function than asymptomatic controls living in low-risk villages. Neuropathologic evaluation of brains from affected Chamorros revealed almost complete destruction of the anterior olfactory nucleus and the presence of high levels of neurofibrillary tangles in related cortical regions. Overall, these findings suggest that the olfactory system is an early and heavily damaged brain region in this disease complex and that these disorders may be caused by exposure to as-yet unidentified airborne environmental agents that enter the CNS through the olfactory pathways.

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Expression of Cell Surface Molecules in Rat Taste Cells Depends Upon Their Innervation. DAVID V. SMITH, MICHAEL T. SHIPLEY (University Cincinnati College of Medicine), and RICHARD A. AKESON (Cincinnati Children's Hospital)

Mammalian taste receptors are modified epithelial cells which are continually replaced throughout life. These cells are arranged within taste buds that are trophically dependent upon their innervation by gustatory nerves. Previous studies have reported the presence of growth-dependent neural membrane proteins on rat taste cells (Finger et al., 1987). Cell-adhesion molecules are important for cell-cell and cell-substrate contacts, which guide development and regeneration in a number of neural systems. Monoclonal antibodies (Mabs) directed against neuronal cell-adhesion molecules (NCAMs) were used for immunocytochemical study of taste buds in the rat vallate and fungiform papillae. Mab 3F4 recognize an amino acid sequence on all three major size classes of NCAM (Akeson et al., 1988). Carbohydrate groups common to NCAMs and other molecules are recognized by Mabs 2B8 and 9OE. In adult normal rats, vallate taste buds contained immunoreactivity for 2B8, 3F4 and 9OE, with a pattern of staining specific to each Mab. Subsets of taste cells were stained with 3F4 and 9OE, whereas 2B8, which also stains a subset of rat olfactory receptor neurons (Allen & Akeson, 1985), appeared to be associated with all of the cells within each taste bud. Taste buds in the fungiform papillae reacted similarly to vallate taste buds to Mab 2B8, but individual cells rarely expressed the antigen to Mab 3F4. However, both intragemmal and perigemmal fibers in the fungiform papillae were strongly reactive to 3F4. Bilateral transection of the glossopharyngeal nerve resulted in the complete elimination of vallate taste cell immunoreactivity to each of these Mabs after seven days. Earlier studies have shown that all morphologically detectable taste buds in the vallate papilla degenerate within this time (Guth, 1957). Studies are underway to determine the recovery of antigenic expression following reinnervation, and the role(s) of these antigens in the normal development of taste cells.

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Localization of Synaptophysin Immunoreactivity in Rat Lingual Tissue. GINA M. NELSON and THOMAS E. FINGER (Dept. of Cell. & Struct. Biol. and Rocky Mountain Taste and Smell Center, Univ. of Colo. Health Sciences Center, Denver, Co. 80262)

Taste buds contain short (axonless) receptors called taste cells. These receptors synapse with intragemmal sensory nerve fibers which enter the taste bud at the basal pole. The receptor cells are short-lived, being replaced by dividing basal cells. In order to investigate taste cell turnover, neuronal innervation, and intercellular communication during growth and maturation, we utilized immunohistochemical techniques to study the distribution of synaptophysin, a synaptic vesicle-associated integral transmembrane protein. Synaptophysin-like immunoreactivity (Syn-LI) was observed at several sites in the rat lingual tissue. In both circumvallate and fungiform taste buds, Syn-LI fibers were seen at the basal pole and extending upward between the receptor cells. Some Syn-LI fibers reached almost to the apical surface of the taste bud. Syn-LI was seen in the nerve processes coursing in the dermal layer adjacent to the taste buds, filling many neural processes near each taste bud's basal pole. Syn-LI was also seen in neural processes in association with lingual epithelium, glands, and muscles. At the ultrastructural level the reaction product is distributed throughout the cytoplasm of the nerve processes, but is heaviest around small vesicles. Large vesicles are not outlined by reaction product. While taste cells were observed at light microscopic levels to be outlined by reaction product, up to this point, Syn-LI has not been observed at ultrastructural levels in the taste cell-neuronal synapses. This distribution is nearly identical to that described for synapsin in rat lingual tissue (Finger, et al., *J. Comp. Neurol.*, in press), another synaptic vesicle protein. Since taste cells (like other axonless receptors) have no requirement for long distance transport of vesicles, these proteins may not be a necessary element of the vesicle membranes.

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Keratin 19-like Immunoreactivity is Specific to Fusiform Cells of Taste Buds. BRUCE OAKLEY, ANNE LAWTON, YOSHIKI SHIBA[1], AND LIANNA WONG (Dept. Biology, Univ. of Michigan; and [1]Dept. Oral Biology, Hiroshima Univ. Dental School)

Diverse and abundant, keratins represent the dominant class of the intermediate filaments of the cell cytoskeleton. Nineteen soft keratin polypeptides have been cataloged in humans, yet the roles of these keratins in epithelial cells remain the least well understood of any of the cytoskeletal proteins. Since each class of reactive human cells produces a limited and characteristic set of keratins, keratin function seems to be closely adjusted to the particular circumstances of each cell type. We report here that a monoclonal antibody specific to human keratin 19 (MAb 4.62) reacted with the cells of all mammalian taste buds tested (rat fungiform, foliate, vallate, naso-incisor and palatine; rabbit fungiform and foliate) but did not react with taste buds of catfish barbel (*Corydoras aeneus*). Each mammalian taste bud had many immunoreactive elongated fusiform cells whose cytoplasm was heavily stained by reaction products of the avidin peroxidase complex bound to a biotinylated secondary antibody. There was little or no immunoreactivity in other lingual epidermal cells. However, luminal cells in the ducts of subjacent salivary glands were reactive. To confirm our impression that MAb 4.62 reacted only with fusiform cells of taste buds, we used MAb KL-1, that reacted only with the basal cells of rabbit lingual epithelium, to show that the keratin 19 epitope is not expressed in basal cells surrounding taste buds, and hence not expressed in the stem cells for taste buds. After denervation there were no immunoreactive cells in rat vallate or foliate gustatory epithelium. This provides additional evidence that the taste bud stem cells that persist after denervation and mediate taste bud regeneration do not express the keratin 19 epitope. The keratin 19-like immunoreactivity of taste buds is interesting: i) because it may serve as a marker of differentiated taste cells, ii) because it points to the close relationship between gustatory and salivary tissue, and iii) because its restriction to fusiform taste cells runs counter to the rule in human tissues that keratin 19 is expressed in basal cells but not in differentiating suprabasal cells.

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Transneuronal labeling of taste receptor cells by carbocyanine dye applied to peripheral gustatory nerves. B. BÖTTGER & T.E. FINGER (Rocky Mountain Taste & Smell Center, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262).

The fluorescent carbocyanine dye, DiI, can be used to trace neural connections in either living or fixed tissue. The dye diffuses within the lipid layers of cell membranes to label all of the processes of any cell to which it has been applied. In both embryonic and adult systems, the dye is known to cross from one cell to another at points of close cell contact, i.e. synapses and gap junctions. We applied diI to peripheral gustatory nerves in aldehyde-fixed mice and ictalurid catfish. The dye was permitted to diffuse for 7-40 days at 20° to 37° C. The mouse circumvallate papillae and the catfish barbels then were sectioned at 20-50 μm on a vibratome. Selected sections were exposed to fluorescent light in the presence of a diaminobenzidine solution thereby producing an osmiophilic reaction product. These photoconverted sections then were osmicated and embedded in Epon for routine electron microscopy.

In both species, diI-labeled nerve fibers formed a dense plexus at the base of taste buds. Other diI-labeled nerve processes could be found within the epithelium near the taste buds. At longer diffusion times, the diI label was present in some taste cells as well as the intragemmal nerve fibers. The labeled taste cells have the classical morphology of receptor cells, extending from the base of the taste bud to the luminal pore. Most taste buds that contained a heavily labeled basal nerve plexus also contained one or more labeled taste cells. Often, labeled taste cells were grouped together within a single taste bud. Ultrastructural analysis shows that the labeled nerve fibers are filled with an electron-dense reaction product. Analysis of the taste cell-to-nerve fiber contacts between labeled processes is underway.

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Elevated Calcium Dependent ATPase Activity of Plasma Membranes of Normal and Denervated Fungiform Taste Bud Cells. M.A.BARRY, L.D.SAVOY (Dept. of BioStructure and Function, Univ. of Connecticut Health Center, Farmington, CT 06032)

These experiments are part of a study on the trophic dependence of taste bud cells on their nerve supply. Vallate and foliate taste bud cells show intense calcium dependent ATPase (Ca^{2+}-ATPase) activity but fungiform papillae have not been previously examined. Ca^{2+}-ATPase activity was revealed histochemically in 10-20 μm sections of the tongue in adult male golden hamsters. Normal fungiform taste buds and the connective tissue core (including nerve fibers) show intense activity relative to the remainder of the fungiform papillae and filiform papillae. Electron microscopy reveals that the Ca^{2+}-ATPase is associated with the plasma membrane of mature taste bud cells, where it probably has a role in maintaining low intracellular calcium in the face of calcium influx related to transduction or synaptic vesicle release. Peripheral "stem" taste bud cells have near to background activity.
The combined chorda tympani-lingual nerve was cut and devitalized unilaterally. In some cases the chorda tympani nerve on the opposite side was also cut. At 7 and 21 days following denervation, the taste buds are smaller than normal but still show high CA^{2+}-ATPase activity. By 21 days there are also 25-40% fewer taste buds than normal. The persistence of denervated taste buds is not due to reinnervation by contralateral nerves. Trigeminal innervation may weakly enhance the persistence of chorda tympani denervated taste buds. These results confirm that denervated fungiform taste buds persist, and that they retain at least one property of normal mature taste bud cells, elevated Ca^{2+}-ATPase activity.

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Quantitative Analysis of Mitoses Inside Taste Buds. E.M.GRANGER AND L.M. BEIDLER (Florida State University)

The current theory on the renewal of cells within taste buds is that epithelial stem cells in a germinal layer surrounding the taste buds divide and some of their daughter cells enter the taste bud, thus replacing those taste cells that are dying (e.g., Beidler and Smallman, '65). Mitotic figures have been seen inside taste buds, but their occurrence has usually been described as rare. Recently high voltage electron micrographs depicting mitotic figures inside taste buds have been presented (Delay, Kinnamon and Roper, '86). However, a quantitative analysis on their frequency has not been presented.

During the course of an extensive light microscopic analysis of serial sections through mouse (C57Bl/6) circumvallate and fungiform taste buds for an unrelated study, we decided to record the number of mitotic figures observed inside these taste buds. All mitotic stages (prophase through anaphase) that could be reliably recognized were counted. In the fungiform taste buds mitotic figures comprised a mean of 0.10% (S.E.=0.0001%) of the total number of taste cells counted in 40 mice (46,002 taste cells). In the circumvallate they comprised 0.03% (S.E.=0.0004%) of the total number of taste cells counted in 72 mice (192,040 taste cells). Most of these mitotic figures were just inside the basal lamina at the base or sides of the buds.

Because the average lifespan of taste cells is long (8-10 days), it is not unreasonble to suggest that even this small percentage of mitoses could account for the turnover of taste cells if one assumes a mitotic duration of one to two hours (Schneiderman, M., personal communication). The presence of mitotic figures inside taste buds does not necessarily mean that the stem cell resides in the taste bud. Epithelial stem cells in various tissues have been shown to produce daughter cells which undergo one or more so-called 'transit divisions' before terminally differentiating. The mitotic figures inside the taste buds could be of this type. The occasional observation of mitotic figures pushing in on or straddling the basal lamina supports this notion.

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Observations of Taste Pore Degeneration in Living Rabbits. ROBIN F. KRIMM and INGLIS J. MILLER, JR. (Bowman Gray School of Medicine, Wake Forest University)

Visualization of the fungiform papilla surface allows for the quantification of taste pores in individual papillae over time. Our objective is to observe the same fungiform papilla before and after the chorda tympani (CT) nerve is lesioned to study the process of degeneration of taste pores in living rabbits. We want to recognize degenerating fungiform papillae and taste pores from the surface and determine the time course of degeneration. Rabbits are anesthetized with Halothane, and the ventral surface of the tongue tip is stained with methylene blue. Images of the tongue are recorded on videotape 7 days before and after the right CT is lesioned in the middle ear. The papillae are mapped, and taste pores are identified on the surface of each fungiform papilla. Typical fungiform papillae have a smooth, unstained surface with a dark border. After surgery, the entire papillary surface of some papillae (42%) appears dark and rough with indentations. The CT-X side of the tongue had 37 of 52 (71%, R=3) altered papillae, while the intact side had 5 of 47 (11%, R=3). Papillae were observed with 1-14 pores before surgery, with a mean of 5.01 (n=86 pap., R=4). After surgery, pores were apparent on 12 of the 42 altered papillae (range 1-4 pores). Of the papillae well-visualized (with pore counts) before and after surgery (n=30, R=2), the altered ones (n=14) had from 4-14 fewer pores. All unaltered papillae contained pores, and in 3 there was a reduction of 3 or more pores. Experiments are underway to study regeneration of taste pores after CT nerve crush. Supported by NIH Grant NS 20101.

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Ultrastructure of Rabbit Fungiform Taste Buds. ANDREW J. BARBER, SUZANNE M. ROYER and JOHN C. KINNAMON. (University of Colorado, Boulder, CO 80309 and The Rocky Mountain Taste and Smell Center, Denver, CO 80262)

In the present study we have used a combination of serial section analysis and high voltage electron microscopy to examine the fungiform taste buds of the rabbit in order to learn how these taste buds and their synaptic connections differ from rabbit foliate (Royer *et al.*, AChemS XI) and circumvallate taste buds (Gill *et al.*, AChemS XII). Our preliminary results generally confirm earlier studies with regard to the general architecture of the rabbit fungiform taste bud, but our results disagree with previous studies with respect to synaptic connections. **Cell Types:** Because the range of cellular morphologies appears to be smaller in fungiform taste buds, it was difficult to classify these cells into types. Rabbit fungiform taste buds are composed of type I, type II, type III and basal cells. The type I cell envelops other cells with cytoplasmic extensions. Type II cells exhibit a range of electron density from electron-lucent cytoplasm and nuclei to moderately dense nuclei and cytoplasm. Some type II cells have unusually electron-dense nuclei. Type III cells are similar in electron density to type II cells but also contain dense-cored vesicles in their nuclear regions. **Synapses:** As expected, we observed synapses between type III cells and nerve fibers. Significantly, we learned that type III cells were not the only taste cell type that formed synaptic contacts with nerve fibers. We also found that electron-lucent cells resembling type II cells also form synaptic contacts with nerve fibers. This heterodox observation is interesting because prior studies concluded that only type III cells formed synapses in rabbit taste buds. The synapses associated with these type II cells are characterized by large aggregations of clear vesicles amassed either in columns or clouds at the active zones. The numbers of vesicles associated with these unusual synapses are far greater when compared with type III cell synapses. The significance of this second putative receptor cell and its unusual synapses is unknown.

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Ultrastructure of Rabbit Circumvallate Taste Buds. JOHN C. GILL, SUZANNE M. ROYER and JOHN C. KINNAMON. (University of Colorado, Boulder, CO 80309 and Rocky Mountain Taste and Smell Center, Denver, CO 80262)

The organization and ultrastructure of cells in the circumvallate taste buds of the rabbit have been examined using high voltage electron microscopy and serial section analysis. Previously, taste cells from the circumvallate papilla of the rabbit have been classified into three distinct types: types I, II and III (plus basal cells). Our preliminary findings are generally consistent with earlier studies, but we believe that there may be more than three cell types in rabbit circumvallate taste buds. Using nomenclature proposed by Royer *et al.* (*AChemS X*) we have subdivided type I cells into types "**I-D**" and "**I-L**," and identified a subtype of the type II cell, which we refer to as "**II-VL**." Taste cell types **I-D** and **I-L** preserve many structural similarities with the classical type I taste cells, such as the presence of cytoplasmic processes that envelop and separate other taste cells and nerve fibers, electron-dense granules in the apical cytoplasm, and apical microvilli. The **I-D** subtype has an irregularly-shaped nucleus and densely staining cytoplasm often containing numerous distended cisternae of smooth endoplasmic reticulum (SER). Type **I-L** cells exhibit cytoplasm of intermediate electron-density containing lamellar or spiraling arrays of closely packed rough endoplasmic reticulum. The **II-VL** cell subtype, usually found toward the periphery of the taste bud, is a large cell characterized by a large round, electron-lucent nucleus and highly vesiculated, electron-lucent cytoplasm. We have also observed, but have yet to classify, a cell that is characterized by an ovoid, lightly-staining nucleus and an electron-dense cytoplasm containing numerous distended cisternae of SER. To date, only type III cells have been observed to make synaptic connections with nerve processes in rabbit circumvallate taste buds. These type III cells typically possess dense-cored vesicles distributed throughout the cytoplasm except in the most apical regions. It is unclear whether the morphological variations observed in taste cells of rabbit circumvallate taste buds represent different cell lines, stages of development of a single cell line, or both.

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Characteristics of the Amiloride-sensitive Na^+ Channel in Taste Cells: Results from Computer Simulation. SHEELLA MIERSON (Medical College of Virginia, Virginia Commonwealth University, Dept. of Physiology, Richmond, VA 23298, U.S.A.)

Direct characterization of apical Na^+ channels in mammalian taste cells is difficult. It is possible to infer some characteristics from a mathematical model of the taste cell. A model, based on network thermodynamics and consistent with usual epithelial topology, was developed for the rat tongue epithelium (M.L.Fidelman & S.Mierson., *Am.J.Physiol.*, *257*:G475-G487, 1989). The model includes amiloride-sensitive Na^+ channels in the apical membrane, and K^+ channels and Na^+-K^+ pumps in the basolateral membrane. Steady-state output variables were calculated for a range of NaCl concentrations in the luminal solution. Simulations indicate that the Na^+ channels in taste cells must have different properties than those typical in tight epithelia such as frog skin. The apical Na^+ channel is described by an equation for a one-site, two-barrier channel. In order for the taste cell to depolarize as the salt concentration increases from 150 to 1000 mM, either (1) the dissociation constant for the Na^+ channel must be an order of magnitude higher than the usual value (E.Mintz, S.R.Thomas, & D.C.Mikulecky, *J.Theor.Biol.*, *123*:21-34, 1986), or (2) the number of channels must increase as salt concentration increases. We have chosen a model with a fixed number of channels of two different types. One is amiloride-sensitive and saturates at high salt concentration (dissociation constant ~300 mM); the other is amiloride-insensitive and saturates at low concentration (dissociation constant ~5 mM). Simulations show that the percentage of Na^+ flux that is sensitive to amiloride increases with salt concentration. This is consistent with data from *in vitro* transport experiments (J.A.DeSimone, G.L.Heck, S.Mierson, & S.K.DeSimone, *J.Gen.Physiol.*, *83*:633-656, 1984) and from neurophysiological measurements (J.A.DeSimone & F.Ferrell, *Am.J.Physiol.*, *249*:R52-R61, 1985). Modeling constrains the parameters of the system, and suggests future experiments.

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Protein Composition of the Von Ebner Gland Secretions. JOHN L. BEIDLER(Florida State University, Tallahassee, Florida)

Studies by Whitney and others have demonstrated a genetic relationship between bitter tasting sucrose octaacetate and the gene for proline-rich proteins, a major salivary protein. Another study has recently shown a relationship between the sublingual gland and sodium taste response. With a renewed interest in the relationship between saliva and taste, we have begun a study of the Von Ebner gland secretions and their relationship to taste. Anatomically, ducts from the von Ebner gland inundate the walls of the circumvallate papillae, with duct openings frequently adjacent to taste buds. On occasion, taste buds have been found in the duct. Biochemical analysis using two dimensional gel electrophoresis of von Ebner salivary secretions reveal greater than 100 proteins present. Western-blot and ELISA techniques have detected the following proteins present in von Ebner secretions; amylase, lingual lipase, ferritin, lactalbumin, nerve growth factor, epidermal growth factor, proline-rich proteins, IgG, IgA, and IgM. Thus, the von Ebner gland appears to be a unique gland, very complex in its secretory properties. This suggests that its function is much more complex than previously suggested and may play a role in taste perception and taste bud maintenance and renewal.

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Evidence for Ins(1,4,5)P_3 as a Second Messenger in Rat Taste Receptor Cell Signal Transduction. P.M. HWANG, A. VERMA, D.S. BREDT, C. ROSS AND S.H. SNYDER (Depts. of Neuroscience, Pharmacology and Molecular Sciences, Johns Hopkins Univ. Sch. of Med.).

Denatonium, a potently bitter substance, has been shown to mobilize intracellular Ca^{2+} in isolated rat circumvallate papilla (CVP) taste receptor cells (Akabas, M.H., et. al., Science, 242:1047, 1988). We examined the energy-dependent sequestration of $^{45}Ca^{2+}$ into 10 um frozen sections of rat CVP via autoradiography. Accumulated $^{45}Ca^{2+}$ was selectively localized to circumvallate papillae, which mediate bitter taste. The Ca^{2+} taken up was taste bud cell-specific as demonstrated by lack of uptake post-denervation. In the presence of 100 nM Ins(1,4,5)P_3 there was a significant reduction in the net accumulation of $^{45}Ca^{2+}$. This effect was IP_3 isomer-specific: Ins(1,4,5)P_3 = Ins(2,4,5)P_3 >> Ins(1,3,4)P_3. Other inositol phosphates such as IP_1 or IP_2 had no effect. The effect of IP_3 on Ca^{2+} uptake was reversed with 100 ug/ml heparin, a known antagonist of IP_3 receptors. In situ/ligand autoradiographic data and biochemical data for the involvement of phosphoinositides in rat taste cell signal transduction are presented.

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Structure/Activity Studies of Alanine and Arginine Taste Receptors in Channel Catfish. KATERINA LEFTHERIS, BRUCE P. BRYANT, JOSEPH G. BRAND (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104, U.S.A.)

L-Alanine (L-Ala) and L-Arginine (L-Arg) bind and activate independent taste receptor sites of the channel catfish. In an effort to develop novel stimuli (agonists), inhibitors (antagonists) and photoaffinity labels to channel catfish taste receptors, a number of analogs of both L-Ala and L-Arg have been prepared and tested for activity in a competitive ligand binding assay, using radiolabeled L-Ala or L-Arg, and in a taste neural preparation. Analogs were developed by modification of the carboxylic acid, alpha amine and side chain of the progenitor amino acids. For alanine analogs, competitive binding assays showed that substitution of the carboxylic acid with substituted benzyl esters led to weak IC50 values compared with L-Ala. Substituted amides, benzyl amides and anilides of L-Ala were also less active in the binding and neural assays as were analogs containing side chain modifications. Analogs modified at the alpha amine were virtually inactive at the alanine binding site. For L-Arg derivatives, all substitutions off the guanidinium group of L-Arg led to weak IC50 values. Octopine (N-alpha-(1-carboxyethyl)-L-Arg), an analog of L-Arg where the alpha amine is extended, showed an IC50 value only slightly poorer than that found with L-Arg in the competitive binding assay, and its neural response was comparable to that elicited by L-Arg. These results indicate that some modifications off the alpha amine of L-Arg may be tolerated and suggest possible routes for developing photoaffinity labels for the L-Arg site.

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Molecular Recognition: A Quantum Mechanical Study of Amiloride Analogs. C.A. VENANZI, C. PLANT (Chemistry Div.,New Jersey Institute of Technology, Newark, NJ 07102), T.J. VENANZI (Chemistry Dept., College of New Rochelle, New Rochelle, NY 10805)

Amiloride is a potent inhibitor of sodium transport in a variety of cellular and epithelial transport systems. Several laboratories have used amiloride analogs to probe the chemical features responsible for the molecular recognition of amiloride at the channel binding site. In the frog skin, the structure-activity data of Li, et. al.[1] relates changes in the substituent pattern on the pyrazine ring or guanidinium side chain to changes in kinetic data for the formation of the amiloride-channel encounter complex or for release of the blocking complex. In order to interpret the activity of these amiloride analogs at the molecular level, it is important to analyze the structural and electrostatic features that determine the optimal interaction of the molecule with the channel binding site. As a first step in this direction, we calculate the quantum mechanical molecular electrostatic potential (MEP) pattern of several amiloride analogs in an attempt to correlate changes in the molecular charge distribution upon binding to differences in the kinetic data of Li, et. al. From inspection of the MEP patterns of the protonated species, we identify features of molecular recognition that may be involved in the formation of the encounter complex. From inspection of the MEP maps of a simple model encounter complex, we identify features that may influence the stability of the blocking complex.

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1.(a) Li,J.H.-Y.; Cragoe, Jr.,E.J.; Lindemann, B. J. Membrane Biol. 1985 83,45;(b)ibid. 1987 95, 171.

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Inhibition of taste responses to Na^+ salts by epithelial Na^+ channel blockers in gerbil. SCHIFFMAN, SS, SUGGS, MS, CRAGOE, EJ Jr., and ERICKSON, RP (Duke University)

The Na^+ transport inhibitor amiloride blocks taste responses to NaCl by 60-70%. The purpose of the present study was to determine if greater inhibition of NaCl and other sodium salts could be achieved with three potent amiloride analogs that are specific for the epithelial Na^+ channel: phenamil, 2',4'-dimethylbenzamil, and 3',4'-dichlorobenzamil. The K_i values for the epithelial Na^+ channel for amiloride, phenamil, 2',4'-dimethylbenzamil, and 3',4'-dichlorobenzamil are 0.34uM, 0.086uM, 0.071uM, and 0.01uM respectively. Application of phenamil (100uM) to the anterior tongue blocked integrated responses to 0.2M NaCl from the chorda tympani nerve by 98.04% but had no significant effect on 0.2M sucrose or 0.2M NH_4Cl. This finding suggests that the epithelial Na^+ channel alone transduces the taste of NaCl in gerbil. The residual 30-40% of the response that is not blocked by amiloride can simply be explained by the fact that amiloride is less potent than phenamil. The epithelial Na^+ channel blockers also suppressed the neural activity elicited by sodium bromide, sodium acetate, monosodium glutamate, sodium bicarbonate, sodium ascorbate, sodium phosphate monobasic, sodium phosphate dibasic, sodium tartrate, sodium succinate, and sodium citrate. On average, 100uM phenamil blocked responses to Na^+ salts with a variety of anions by 94.2%; 100uM 2',4'-dimethylbenzamil, by 89.83%; and 100uM 3',4'-dichlorobenzamil, by 72.56%. None of the epithelial Na^+ channel blockers affected responses to NH_4Cl or sucrose. Small residual responses to sodium salts of glutamate and phosphate were not eliminated by the amiloride analogs; this suggests that other transduction mechanisms may account for a portion of taste responses for these salts in the gerbil.

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The effect of amiloride analogs on taste responses in gerbil. SCHIFFMAN, SS, FREY, AE, SUGGS, MS, CRAGOE, EJ Jr., and ERICKSON, RP (Duke University)

The discovery that transport of sodium ions is involved in salty taste perception was made by DeSimone et al. (Science 214: 1039, 1981) and Schiffman et al. (Proc. Natl. Acad. Sci. USA 80: 6136, 1983) who used the sodium ion transport inhibitor amiloride to block responses to NaCl. Subsequent neurophysiological and transport studies have confirmed these results. Amiloride and its analogs have been found to inhibit three types of Na^+ transport systems in a variety of epithelial preparations: the epithelial Na^+ channel, the Na^+/H^+ exchanger, and the Na^+/Ca^{++} exchanger (Kleyman and Cragoe J. Mem. Biol. 105: 1-21, 1988). The purpose of the present study was to apply amiloride analogs with different affinities for these three types of transport systems to the tongue of gerbil to determine which of these Na^+ transport mechanisms mediates salt taste. The effects of 8 amiloride analogs on electrophysiological taste responses to NaCl, $CaCl_2$, sucrose, and glutamic acid were assessed. The pattern of responses from the chorda tympani nerve indicates that the taste of NaCl is almost totally accounted for by the epithelial Na^+-channel. Phenamil, an amiloride analog which specifically blocks the epithelial Na^+-channel at low concentrations, suppressed the taste responses to 0.03M NaCl by 97%. The pattern of responses also indicates that the Na^+/H^+ antiporter and the Na^+/Ca^{2+} exchanger do not mediate salt taste in the gerbil. None of the amiloride analogs blocked taste responses to $CaCl_2$, sucrose, or glutamic acid. It is concluded that the salty taste of NaCl is almost totally mediated by the epithelial Na^+-channel in the gerbil, and the kinetics of this channel are identical to amiloride-sensitive sodium channels in other systems.

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L-Proline Activates Cation Channels Different from Those Activated by L-Arginine in Reconstituted Taste Epithelial Membranes from Channel Catfish. TAKASHI KUMAZAWA, JOHN H. TEETER, AND JOSEPH G. BRAND (Monell Chemical Senses Center, University of Pennsylvania and Veterans Administration Medical Center, Philadelphia, PA 19104)

Abstract will be available at the meeting.

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Two Types of Arginine-Best Taste Units in the Channel Catfish. J. KOHBARA, S. WEGERT and J. CAPRIO. Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA. 70803-1725.

The facial taste system of the channel catfish, *Ictalurus punctatus*, is highly sensitive to amino acid stimuli (Caprio,'75;'78). The L-isomers of arginine and alanine are the most stimulatory amino acids and bind to independent receptor sites (Caprio,'82; Cagan,'86; Kalinoski et al.,'89). The distribution of these sites across the population of taste cells is heterogeneous as alanine-best (ALA) and arginine-best (ARG) taste units comprise the two major types of amino acid-sensitive fibers (Caprio and Tucker,'76; Davenport and Caprio,'82). A quantitative investigation of 57 facial taste units that innervate maxillary barbel taste buds indicated that 33 were ALA and 24 were ARG units. The ALA units responded with highest frequency to L-alanine, but also responded to equimolar concentrations of other amino acids. The ARG units were of two types, those that were narrowly-tuned to L-arginine (n=7), and those that were also responsive to 1 mM L-proline (n=17). Interestingly, three of the proline-sensitive ARG units became proline-best at 1mM stimulus concentrations. The ALA units were relatively insensitive to L-proline. Independent, low affinity L-proline receptor sites were previously indicated in both the facial (Wegert and Caprio, AChemS'88) and glossopharyngeal-vagal (Kanwal and Caprio,'83) taste systems of the channel catfish. The present report indicates that proline information is transmitted primarily by a subset of arginine-sensitive neurons and suggests the existence of at least three different types of amino acid-sensitive taste cells in the channel catfish: (a) taste cells that contain primarily alanine receptor sites, (b) taste cells that contain primarily arginine receptor sites, and (c) taste cells that contain primarily proline receptor sites, or taste cells in which both arginine and proline sites are co-expressed.

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Garter Snake Accessory Olfactory Bulb Neurons Respond to a Chemoattractive Protein Purified from Earthworm Secretions. JUN INOUCHI, XIAN-CHENG JIANG, DALTON WANG, JOHN KUBIE and MIMI HALPERN (State University of New York, Health Science Center at Brooklyn).

A proteinaceous chemoattractant for garter snakes (Thamnophis sirtalis) was purified by means of Bio-gel P2-column chromatography and semi-preparative native polyacrylamide gel electrophoresis from earthworm, Lumbricus terrestris, electric shock-induced secretion. The chemoattractant is a glycoprotein with an apparent molecular weight of about 20kDa (Jiang et al., Chemical Senses 12:667, 1987). The chemoattractant binds to snake vomeronasal sensory epithelial membrane fractions but not to membrane extracts of the non-sensory epithelium of the vomeronasal mushroom body (Jiang et al., Chemical Senses 13:749,1988). Electrophysiological recordings revealed that single neurons in the accessory olfactory bulb of garter snakes markedly changed their firing rates when the 20kDa chemoattractant was delivered to the sensory epithelium of the snake vomeronasal organ. No changes in firing rate were observed when the epithelium was exposed to control, blank polyacrylamide gel eluate. Most neurons increased their firing rate, although some decreased their firing rate, in response to liquid delivery of the 20kDa protein to the vomeronasal epithelium. The change in firing rate was concentration-dependent. This is the first clear electrophysiological demonstration of vomeronasal system response to a purified, biologically significant non-volatile chemical stimulus.

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IMMUNOLOGICAL ANALYSIS OF CHEMOATTRACTANTS FROM EARTHWORM TO GARTER SNAKES. DALTON WANG, PING CHEN, AND MIMI HALPERN (State University of New York, Health Science Center at Brooklyn).

Garter snakes (Thamnophis sp.) respond to objects covered with surface washes and secretions of earthworms (Lumbricus terrestris) with characteristic tongue flicking and attack. This discriminated response is mediated by the vomeronasal system. Several proteinacious chemoattractants have been purified from earthworm wash (Wang et al., Arch. Biochem. Biophys. 267:459, 1988) and electric shock-induced earthworm secretions (Jiang et al., Chemical Senses 12:667, 1987). Polyclonal antibodies for two purified chemoattractants derived from earthworm wash and electric shock-induced earthworm secretion were raised in New Zealand rabbits. These antibodies are immuno-specific for their respective antigens and do not cross-react with each other. However, each antiserum does cross-react with other chemoattractants present in either earthworm preparation to varying degrees. This phenomenon was further evidenced by the nonuniformed removal of various chemoattractive proteins by their respective antisera from each earthworm preparation, as revealed by polyacrylamide gel electrophoresis. These results have also been confirmed with purified chemoattractants. Among the 5 isolated proteins (excluding the two antigens), only one (the band 6 protein from earthworm wash) did not cross-react with either antiserum. The chemoattractivity of the purified proteins (antigens) could not be neutralized by reacting with their respective antisera, suggesting that the antigenic determinant (the epitope) of the antigen (chemoattractive protein) is not an integral part of chemoattractive domain. At least, this is true for the large population of the polyclonal antibodies.

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Projections of the terminal nerve to the pterygopalatine ganglion in voles, Microtus ochrogaster. CELESTE R. WIRSIG-WIECHMANN (Wake Forest University) and JOHN J. LEPRI (University of North Carolina at Greensboro)

The terminal nerve (TN) appears to be involved in the chemosensory stimulation of reproduction: it is closely associated with the vomeronasal system and exhibits luteinizing hormone-releasing hormone-immunoreactive (LHRH-ir) material. How the TN interacts with nasal structures is unclear. We examined the LHRH-ir component of the TN in voles, a species that relies heavily on chemosensory cues for reproduction. Robustly labeled perikarya and fibers extended rostrally to contact LHRH-negative ganglia and glands in the vestibule of the nasal cavity. Small groups of LHRH-ir perikarya and fibers were seen from the base to the surface of the sensory epithelium in the vomeronasal organ (VNO) and within the vomeronasal nerves. Labeled fibers were also seen in the nasopalatine nerves and in the pterygopalatine ganglia (PPG). The PPG contained a dense plexus of LHRH-ir fibers and cell bodies. A few LHRH-ir fibers were seen in the trigeminal nerves and ganglia. Labeled fibers from the nasal cavity joined a plexus of labeled perikarya and fibers on the medial surface of the olfactory bulbs. From this plexus, fibers extended to the accessory olfactory bulbs and around the entire circumference of the caudal olfactory bulbs. Two large bundles of LHRH-ir fibers penetrated the brain caudal to the olfactory bulbs and projected to groups of LHRH-ir cell bodies within the brain. The unique projection of LHRH-ir TN fibers to the PPG in voles suggests that this component of the TN may modulate the access of chemical stimuli to the VNO.

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ACh and NE Effects on Elasmobranch Nervus Terminalis Ganglion Cells: Spectral Analysis and Computer Modelling. JOEL WHITE (Dept. of Neurosurgery, Tufts-New England Medical Center, Boston, MA), MICHAEL MEREDITH (Dept. of Biological Science, Florida State University, Tallahassee, FL).

Previous anatomical studies indicate that the nervus terminalis (NT) ganglion in the bonnethead shark (*Sphyrna tiburo*) contains components of both cholinergic and catecholaminergic neurotransmitter systems (White, 1989, *Soc. Neurosci. Abstr.* #19.5). Preliminary pharmacological studies indicated that both norepinephrine (NE) and acetylcholine (ACh) can reduce spontaneous ganglion cell activity recorded in NT nerve trunks *in vitro* (White and Meredith, 1988, *AChemS IX Abstr.* #38). In the present study, power spectra of multi-unit, whole nerve NT recordings were studied before, during, and after ACh and NE perfusion. The data show that the effect of ACh is variable, producing a decrease in activity and total spectral power in some nerves and an increase in others. A computer model was developed to aid in understanding how changes in power spectra may reflect changes in the firing rates of cells whose axons contribute to the whole nerve activity. The actions of ACh and NE can be explained if there are at least two NT ganglion cell populations, one contributing primarily to high spectral frequencies (narrow spikes) and another to low spectral frequencies (broad spikes). ACh (10 and 100 μM) may have opposite effects on these two populations. The observed decrease in Half Power Frequency (HPF; the median frequency of the power spectrum) with no change in total spectral power averaged over all nerves is consistent with a decrease in narrow spike firing rate (high spectral frequency cells) and an increase in broad spike firing rate (low spectral frequency cells). 10 μM NE reduced total spectral power and increased HPF, consistent with a decrease in broad spike firing rate (low spectral frequency cells) and little or no change in narrow spike firing rate (high spectral frequency cells). In intracellular recordings from NT ganglion cells, the responses of four cells tested with pharmacological agents were consistent with those proposed for the high spectral frequency cells. Carbachol (a cholinergic agonist) elicited a marked hyperpolarization and a reduction in membrane resistance, while NE had no influence on the cells. The physiological findings are consistent with the anatomical observations, and the combined data provide a basis for a proposed circuit of the NT ganglion.

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Vomeronasal systems in salamanders: Comparison across larval and adult phases of species with differing life-history strategies. HEATHER L. EISTHEN, DALE R. SENGELAUB, and JEFFREY R. ALBERTS (Department of Psychology and Program in Neural Science, Indiana University).

Chemosensory stimuli influence both feeding and social behavior in the adult axolotl (Ambystoma mexicanum), a Mexican salamander that does not metamorphose and thus remains aquatic throughout its life. The main olfactory receptors are located in the medial portion of the nasal cavity, and the axons of these receptors terminate in the main olfactory bulb. A separate receptor-lined pouch extends along the lateral aspect of the nasal cavity; this is the typical site of vomeronasal receptors in terrestrial salamanders. The axons of these laterally-located receptors terminate in a distinct accessory olfactory bulb, located dorsal and caudal to the main olfactory bulb. If it is true that the vomeronasal system responds to non-volatile molecules and is therefore well-suited for terrestrial life, then its presence in the aquatic axolotl is paradoxical. We have taken a comparative approach, collecting evidence of vomeronasal systems in different phases of the life-cycle in four salamander species from two families. We find that the aquatic larvae of Jefferson's salamanders (A. jeffersonianum), tiger salamanders (A. tigrinum), and axolotls (A. mexicanum) also all contain a lateral pouch in the nasal cavity and an accessory olfactory bulb in the forebrain, indicating the presence of a vomeronasal system. Adult tiger salamanders are terrestrial but adult red-spotted newts (Notophthalmus viridecens) are secondarily aquatic, having passed through a terrestrial sub-adult (eft) phase. Adult tiger salamanders and red-spotted newts both possess a lateral pouch and accessory olfactory bulb. Bertmar (1981, Evol., 35: 359) hypothesized that the vomeronasal system evolved as an adaptation to terrestrial life. However our data suggest that the vomeronasal system was not originally adapted to terrestrial life, but that its function has changed over time.

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LHRH Injected Intracerebrally, Relieves Some Behavioral Deficits of Male Hamsters after Vomeronasal Organ Lesions. MICHAEL MEREDITH, GAY HOWARD and MARY WISGIRDA (Biological Sci. Dept., Florida State University, Tallahassee, FL 32306

Several lines of evidence suggest that one consequence of vomeronasal afferent input is the release of LHRH in the brain. Other evidence suggests LHRH is facilitatory to mating behavior. We have investigated the possibility that exogenous LHRH might substitute for vomeronasal input in sexually inexperienced male hamsters that show mating behavior deficits after vomeronasal organ (VNO) removal. Sexually inexperienced adult male hamsters were either sham operated (SHAM) or had their vomeronasal organs removed (VNX). At least 4 weeks later, a 26 ga guide tube was implanted stereotaxically with its tip in the lateral cerebral ventricle of each animal. Except during testing, the guide tube was sealed with a screw-on cap and dummy cannula. Mating behavior was tested at 2+ day intervals in clean pastic cages with naturally cycling behaviorally receptive females. Investigation time, mounts, thrusts and intromissions were recorded for 5 min or until the male acheived 5 intromissions. A 2 ul volume of LHRH (50 ng, 6 tests) or saline (6 alternated tests) was injected into the ventricle of each animal 30 min before a test, through a 33 ga cannula, in 3 pulses 30 sec apart using a Picospritzer. VNX animals showed significant deficits in mating behavior compared to SHAM animals when tested after saline injection (0.2 vs 1.6 intromissions/min; p=0.002: Mann-Whitney Test, 2 tail). After LHRH injection, the performance of the two groups was no longer different. As expected under the rather naive hypothesis, VNX animals showed significant improvement (0.7 intro/m p<0.05 Walsh Test). Unexpectedly, the SHAM animals showed significant decline in performance (1.1 intro/m p<0.05 Walsh Test). Overall the 2 groups responded significantly differently to LHRH vs Saline (p<0.05, Mann-Whitney Test). Investigation of the females' anogential region was not significantly changed by LHRH, suggesting its effect is not one of increased non-specific arousal. Studies of doses, latencies and sites of action continue.

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Nuzzling In The Grey Short-Tailed Opossum Delivers Odorants To The Vomeronasal Organ. NAOMIE S. PORAN, ALEXANDRA VANDOROS and MIMI HALPERN (State university of New York, Health Science Center at Brooklyn)

A unique type of chemosensory investigative behavior, termed nuzzling, has been observed in the marsupial *Monodelphis domestica*. Nuzzling is performed when an animal encounters a conspecific scent mark and involves rubbing the ventral aspect of the snout in repeated forward motions over the odor deposit; during nuzzling, the opossums wet the odor deposit with oral fluids. Behavioral experiments have demonstrated that nuzzling is intimately involved in social recognition in this species. Familiarity with conspecifics (both related and unrelated) was found to significantly reduce nuzzling duration. Autoradiographical analysis of snout tissue from animals that nuzzled conspecific odor deposits mixed with ^{3}H-proline in dry form, demonstrated that the chemicals were transported into the vomeronasal organ (VNO) through the nasopalatine canal. The radioactive labeling was highly concentrated in the non-sensory epithelium and to a considerably lesser extent in the supporting cell layer of the sensory epithelium, mainly in its lateral aspect. No radioactivity above background levels was observed in the olfactory epithelium. Several morphological structures such as paired lip grooves, a smooth-muscle upper palate flap, unusually elaborate vascularization and a large gland may be important in the accessibility of odorants to the VNO.

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Neural Cross- and Self- Adaptation of Trigeminal Nerve Responses to a Variety of Chemical Stimuli. L.G. FARLEY, W.L. SILVER (Dept. of Biology, Wake Forest University, Winston-Salem, NC 27109)

Trigeminal chemoreceptors in the nasal cavity respond to a variety of volatile chemical stimuli. However, how the chemical stimulus interacts with the nasal trigeminal nerve ending remains a source of speculation. If different mechanisms exist for the interaction of different types of compounds with the nerve ending then it follows that trigeminal nerve fibers exhibit some degree of specificity. The present study employed electrophysiological, whole-nerve, cross- and self-adaptation paradigms to examine this question. Cross- (self-) adaptation occurs when exposure to one compound lowers the magnitude of the response to exposure of a subsequent different (same) compound. Five stimuli (amyl acetate, AA; l-carvone, LC; d-carvone, DC; l-menthol, ME; and toluene, TO) were used in this study of the rat ethmoid nerve. Each of these stimuli was tested against itself and the 4 other compounds Background concentrations were chosen to to elicit equivalent responses. Results are shown in the table. Symmetrical cross-adaptation occurred between AA and LC, AA and DC, AA and TO, and LC and DC. This suggests that these compounds may elicit responses via similar neural mechanisms. There was no cross-adaptation between ME and TO, implying that different neural stimulating mechanisms exist for these two compounds. There was asymmetrical cross-adaptation between AA and ME, LC and ME, DC and ME, DC and TO, and LC and TO. Asymmetrical cross-adaptation has been suggested to be due possibly to "complex stimulus acceptors" (e.g. Caprio and Byrd, 1984). The results of our study suggest that different compounds may stimulate trigeminal chremoreceptive fibers using different neural mechanisms and that some degree of specificity may exist in these fibers.

as/ts	aa	lc	dc	me	to	
aa	S	X	X	X	X	ts = test stimulus
lc	X	S	X	X	X	as = adapting stimulus
dc	X	X	S	X	X	S = self-adaptation
me	N	N	N	S	N	X = cross-adaptation
to	X	N	N	N	S	N = no cross-adaptation

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The System of Solitary Epidermal Chemoreceptor Cells: A novel Vertebrate Chemosense? KURT KOTRSCHAL (University of Colorado Health Sciences Center, Department of Cellular and Structural Biology, Denver, CO 80262), ROB PETERS (University of Utrecht, Laboratory for Comparative Physiology, NE-3584 Utrecht)*

Solitary chemoreceptor cells (SCC) occur in lampreys, in most actinopterygian fishes and in anuran tadpoles. SCCs are similar in fine structure to taste bud (TB) receptor cells, but come as single, spindle-shaped cells. SCCs are embedded within the epidermis and protrude a single, apical microvillus into the environment (*Nature* 208:703-704). A quantitative SEM survey in 12 teleost species revealed densities from 175 (neon tetra) to 4000 (roach) SCC apices per mm² of skin (*Env.Biol.Fish.* in pr.); in each species, **there were at least twice as many SCCs than TB receptor cells.** Rocklings (Gadidae: Teleostei) show a specialized anterior dorsal fin (ADF), packed with millions of SCCs, which synapse exclusively onto fine-caliber fibers of the recurrent facial nerve (*J.Comp.Neurol.* 268:109-120, *J.Zool.* 216:339-366). Sum potential recordings (*J.Mar.Biol.Assoc.U.K.* 67:819-823) and behavioral experiments (*Biol.Bull.* 177:in pr.) showed narrow tuning of the rockling SCC system to heterospecific fish body mucus. There was virtually no overlap in stimulus spectrum of these SCCs with pelvic fin TBs. By Di-I tracing and HV-EM, the peripheral and central patterns of innervation of SCCs versus TBs are being investigated. The rockling pelvic fin contains both SCCs and taste buds. Application of DiI to either the spinal nerve or the recurrent facial nerve will permit investigation of differential innervation of these systems.

In aquatic vertebrates, SCCs may form a chemosensory input channel, structurally and functionally distinct from conventional taste. If the results from the rockling model can be generalized, the function of SCCs may be bulk water sampling in the context of species interaction rather than localization of food.

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Glutathione Chemoreceptor of Hydra. W. GROSVENOR, S. BELLIS, G. KASS-SIMON AND D.E. RHOADS. (Univ. of Rhode Island, Kingston, R.I.)

Reduced glutathione (GSH) has been demonstrated to be a specific activator of the feeding response in Hydra. GSH, from the prey, induces tentacle concerts and mouth opening that precede ingestion of the prey. Using a bio-assay for the response, the K_d for GSH has been estimated to be 10^{-6}. The feeding response in Hydra can be inhibited with glutamate. This inhibition can be reversed by adding additional glutathione (Lenhoff, H.M. 1968. Behavior hormones and Hydra. Science 161: 434-442). Using a crude membrane fraction of Hydra vulgaris (attenuata) direct radioligand bipding to the putative receptor was done using [^{35}S]-GSH (20nM; 100Ci/mmol). Binding of [^{35}S]-GSH is specific and saturable with a K_d of 9uM and B_{max} = 213pmol/mg protein. Scatchard and Hill analyses are consistent with a single class of binding site. The known agonist, S-methyl glutathione, and antagonist, oxidized glutathione, both inhibit GSH binding to the receptor. Neither glutamate nor the glutamate analogues, quisqualate, kainate, and N-methyl,D-aspartate, at concentrations up to 20uM, inhibit the binding of [^{35}S]-GSH to the receptor. As an inhibitor of the feeding response, glutamate must act noncompetitively at a site that is distinct from the GSH binding site.

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Voltage-dependent Whole-cell Currents in Isolated Fungiform Taste Buds of the Hamster. 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).

Although numerous studies have demonstrated the presence of voltage-dependent ionic currents and electrical excitability in taste cells of lower vertebrates, comparatively few studies have been done on mammalian taste cells. We have recently modified a procedure developed by Behe et al. (I.S.O.T. X, p. 72, 1989) to record whole-cell currents in taste cells from hamster fungiform papillae. To isolate taste buds, hamster tongues were injected with collagenase and the epithelium was gently separated from the underlying connective tissue. Narrow strips of epithelium containing a single taste bud were mounted horizontally in the recording chamber so that the basolateral region of the taste bud cells could be visualized. Individual taste cells were studied with the whole-cell configuration of the patch clamp technique. Most taste cells responded to depolarizing voltage steps with a rapidly-activating, transient inward current followed by a slowly-activating, more sustained outward current. The inward current was blocked by TTX and had properties similar to the voltage-dependent Na^+ current observed in mudpuppy taste cells. Sodium currents in hamster taste cells activated at -40 mV, peaked at 0 mV and were completely inactivated within 5 ms. Na^+ current was half maximal at a holding potential of -60 mV and completely inactivated at a holding potential of -40 mV. The outward current was blocked by TEA and Ba^{++}, indicating that it is a K^+ current. Like K^+ currents in mudpuppy taste cells, K^+ currents in hamster taste cells activated at approximately -10 mV and reached a peak in approximately 30 ms. However, unlike K^+ currents in mudpuppy taste cells, K^+ currents in many hamster taste cells showed voltage-dependent inactivation; K^+ current typically inactivated during long depolarizing pulses with a time constant of about 1 sec was completely inactivated by a holding potential of -20 mV. Some taste cells also contained a component of K^+ current that did not appear to inactivate, resembling the large, non-inactivating K^+ current observed in mudpuppy taste cells. The magnitude of the peak K^+ current varied greatly in different taste cells, ranging from only a few pA in some cells to over 1 nA in others. These data demonstrate that most hamster taste cells are capable of electrical excitability under appropriate conditions. In future studies we plan to examine the influence of taste stimuli on these voltage-dependent currents.

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Identification of Potassium Currents in Rat Taste Cells and Their Modulation by Tastants: Whole Cell Patch Clamp Analysis. **M. SCOTT HERNESS.** (The Rockefeller University, New York, NY 10021)

Modulation of outward currents in taste cells has been proposed as a possible transduction mechanism in taste cells for sour, sweet, and bitter stimuli. Standard patch clamp recording techniques were employed to achieve voltage clamp records in the whole-cell configuration. Cells were held at negative 80 millivolts and stepped at negative or positive 10 millivolt increments. Outward currents ranged from 200 - 800 picoamperes to the largest depolarizing step. Pharmacologic identification of the ionic components of these currents revealed multiple classes of potassium channels. TEA blocked almost all outward current in the cells tested; the remaining current may be non-specific 'leak'. 4AP had similar results. Data thus far suggest that taste cells contain delayed rectifier potassium channels, A-type potassium channels, and probably the Ca-activated potassium channels. The A channel appears to exist only in some taste cells whereas the delayed rectifier appears more ubiquitous. No evidence thus far has been obtained for the M-type potassium channel or for inward rectifier potassium channels.

Tastant solutions, added to the bath perfusate, affected these voltage sensitive potassium currents. Quinine profoundly blocked outward currents, appearing to act mostly on the delayed rectifier and A channels. Theophylline similarly diminished these outward currents. Saccharine was found to block outward currents in some taste cells and to have no effect in others. Data thus far suggest it acts at least on the delayed rectifier channel. In one cell sucrose actually augmented outward currents. These tastant effects were reversible. It is yet not known whether tastants directly effect potassium channels or if their effects are mediated through a second-messenger system.

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The Anion in Mammalian Salt Taste - a Paracellular Hypothesis. ELLEN J. ELLIOTT (Duke University), SIDNEY A. SIMON (Duke University).

The mechanism by which the anion influences salt taste is still unknown. One measure of the influence of the anion is the amiloride sensitivity of the chorda tympani response. That is, the response to different sodium salts is inhibited to different degrees, depending on the anion. We investigated several possible explanations of this. To examine the possibility that some organic Na salts might stimulate differently from NaCl because they decrease intracellular pH (pHi), we measured responses to 0.5M Na acetate at extracellular pH 7.4 (where acetate should change pHi) and 9.2 (where it should not). At both pH's, the responses to Na acetate remained totally amiloride-inhibitable (in contrast with the response to NaCl, which was inhibited only 60-70% by amiloride). This indicates that a change in pHi is not responsible for the difference between Na acetate and NaCl. We also tested inhibitors of anion exchangers (SITS, DIDS), of Cl co-transporters (bumetanide, furosemide), and of Cl channels (9-anthracene carboxylic acid, SITS and an antibody shown to inhibit Cl conductance pathways) on the response to NaCl. None of these inhibitors depressed either the total response or the amiloride-insensitive portion. Thus, there is no evidence that any of these Cl transporting membrane proteins play a role, in the taste cell apical membrane, in salt taste. A hypothesis which is consistent with our data and which can explain a number of experimental observations, is that the response to NaCl involves both a cellular pathway (an amiloride-sensitive Na channel in taste cell apical membranes) and a paracellular pathway (anion-permeable tight junctions between taste cells). Substitution for Cl with other anions that are less permeant through the tight junctions, or through the basolateral membrane, could change the membrane potential of taste cells and thus modulate their synaptic activity.

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The pattern of kinetics for hodulcin suppression of fly receptor cell responses to sucrose is characteristic of an effect on breakdown of a stimulus-receptor complex. DOUGLAS E. KOLODNY and LINDA M. KENNEDY (Dept. of Biology, Clark University, Worcester, MA 01610)

Hodulcin (from Hovenia dulcis) selectively suppresses sweetness perception in humans and behavioral and receptor cell responses to sucrose in flies. The chemistry and physiological effects of hodulcin are similar to those of gymnemic acids and ziziphins (Kennedy et al., Chem. Senses 13, 1988, 529; Kolodny and Kennedy, Chem. Senses 13, 1988, 545), and the three probably act by similar mechanisms. To elucidate the mechanisms, we studied the actions of purified hodulcin (HDE) on Phormia regina receptor cell action potential responses to sucrose (in 50 mM NaCl). The concentration-effects (C-E) curve for responses to sucrose 50 mM is bell-shaped: suppression begins at 0.025% and peaks at 0.075% w/v HDE. There is a significant decrease at 0.1% HDE, but a small, significant suppression remains ($p \leq 0.01$) (Kruskal-Wallis; Mann-Whitney). The curve is similar to C-E curves for gymnemic acids and ziziphins. In kinetic studies, nonlinear regression fits for responses to sucrose 10, 20, 40, 80, 160, and 320 mM to the Michaelis-Menten equation showed a successive decrease in Vmax and successive increase in Km as HDE concentration increased (0.0-0.03-0.07%) ($p \leq 0.04$). However, the effect on Km decreased at higher HDE concentrations (0.1 and 0.2%) ($p \leq 0.03$) (ANOVA). The results indicate mixed (competitive and noncompetitive) kinetics for lower HDE concentrations, and primarily noncompetitive kinetics at higher concentrations. This pattern of kinetics is characteristic of an effect on breakdown of a stimulus-receptor complex and could result from action on a later step in a cascade transduction process and/or a change in the physical chemistry of the membrane. Correspondences between the C-E curve for HDE receptor cell effects and a curve for HDE micelle formation supports a physicochemical mechanism. We have found a similar kinetic pattern for gymnemic acids suppression of sweetness perception in humans (de los Santos et al., this volume).

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The pattern of kinetics for gymnemic acids suppression of human sweetness perception is characteristic of an effect on breakdown of a stimulus-receptor complex. HANNAH C. DE LOS SANTOS, SHARON GREEN and LINDA M. KENNEDY (Dept. of Biology, Clark University, Worcester, MA 01610)

The gymnemic acids (from Gymnema sylvestre) selectively suppress behavioral and afferent neural responses to sweet stimuli in humans and some other vertebrates and behavioral and receptor cell responses to sucrose in flies. The chemistry and physiological effects of gymnemic acids are similar to those of ziziphins and hodulcin; thus the three probably act by similar mechanisms. Kinetic analyses of psychophysical data for gymnemic acids effects suggest competitive or mixed inhibition mechanisms (Kennedy, Chem. Senses, 14(6), 1989). For comparison, we analyzed psychophysical data of Mize and Frank (Chem. Senses 13, 1988, 720) for the effects of G. sylvestre extracts (Gs), distilled water or tea on the sweetness of acesulfame K, aspartame, sodium cyclamate, fructose, glucose, sucrose, stevioside and xylitol. Linear regression of the data on double-reciprocal plots and nonlinear regression fits to the Michaelis-Menten equation gave relationships that fit a noncompetitive model, but statistical results (ANOVA) did not permit competitive or uncompetitive mechanisms to be ruled out. The results suggested mixed mechanisms, primarily noncompetitive inhibition. The kinetic parameters were similar for all the sweeteners; thus a receptor occupancy/blocking mechanism seemed unlikely. Comparison across studies revealed a pattern-- competitive or mixed kinetics at low, and primarily noncompetitive kinetics at high Gs concentrations. This pattern is characteristic of an effect on breakdown of a stimulus-receptor complex and could result from inhibition of a later step in a sequential-step transduction process that is coupled to multiple sweet receptors, and/or, or as a result of, a change in membrane physical chemistry. Surface tension data (DeSimone et al., Chem. Senses, 5, 1980, 317) support a physicochemical mechanism. We have found a similar kinetic pattern for hodulcin suppression of fly receptor cell responses to sucrose (Kolodny and Kennedy, this volume).

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Salt Responses of Lingual Branch of Trigeminal Nerve are Inhibited by Lanthanum. A.L. SOSTMAN (Department of Neurobiology, Duke University) and S.A. SIMON (Departments of Neurobiology and Anesthesiology, Duke University)

The lingual nerve of anesthetized rats was exposed surgically, cut at the level of the foramen ovale and placed over a platinum wire recording electrode. Integrated whole-nerve responses were recorded to stimuli flowing over the tongue through a dam at 2 ml/sec. To control for neural activity evoked by flow onset and thermal changes, an adapting stimulus of distilled water was applied for 25 sec before each test solution of NH_4Cl, NaCl and KCl (2.5 M, pH 7.0, 30°C, for 25 sec). Threshold concentrations for responses to these salts are about an order of magnitude greater than those for the chorda tympani nerve. All three salts produce distinct neural responses. NH_4Cl results in a relatively large and transient (<10 sec) increase in neural activity while NaCl produces a smaller, more prolonged increase. KCl, in turn, produces a smaller and less prolonged increase in activity than NaCl. Repeated application of the salts resulted in progressively smaller responses. In most experiments a decrease in activity preceded the increase. We attributed this decrease to the osmotic pressure of the salt solutions since increases in activity were observed upon rinsing with hyposmotic solutions. To determine if these salts activate lingual nerves via transcellular or paracellular pathways, various pathway inhibitors were used. Incubation of the tongue with $LaCl_3$, an established inhibitor of tight junctions (2.5 mM for 30 sec), attenuated the response to each salt by 40-100%. However, neural responses to cooling, heating or pinching the tongue were not inhibited by lanthanum, a result consistent with a distinct class of trigeminal chemosensory fibers. Lanthanum inhibition was reversible for all salts, most recovery occurring within 20 min. By contrast, incubation either with the epithelial sodium-channel blocker, amiloride (0.1 mM), or with the potassium-channel blocker, TEA (25 mM), did not affect any of the salt responses. These results suggest that in evoking trigeminal responses the salts diffused across the epithelial tight junctions and activated the lingual nerve fibers.

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A New Method for Recording from the Gerbil's Single Chorda Tympani Neurons. LATCHMAN SOMENARAIN and WILLIAM JAKINOVICH JR. (Department of Biological Sciences, Lehman College and the Graduate School, City University of New York, Bronx, NY 10468, USA).

This study presents a new technique for recording electrical activity from single chorda tympani neurons. To do this, we obtained electrophysiological responses from the uncut neurons as they course through the gerbil's middle ear. First, the nerve was pared down to a small strand which was about 10% of its size. Then, a high impedance (1-2 meg ohm) metal microelectrode was inserted into the strand. This was led into a high impedance amplifier and single action potentials were observed on an oscilloscope. The action potentials were recorded onto magnetic tape for later photography or computer analysis. The fungiform taste receptors were stimulated by a continuously flowing system to which taste stimuli were added. The taste stimuli included 0.5M Sucrose, 0.3M NH_4Cl, 0.05M Acetic Acid, 0.01M Quinine HCl, 0.3M NaCl, and 0.3M KCl. Mechanical stimulation was also applied using a small brush. The time to obtain recordings, from administering the anesthetic to seeing action potentials, is about one hour compared to the four hours used by Oakley's laboratory that used the submandibular approach (Cheal et. al, 1977). A disadvantage to the submandibular approach, especially to the novice, are the numerous delicate blood sinuses, which seem to break if one looks at them. Finally, in the submandibular approach the neurons have to be cut before being recorded from, something which may alter their synaptic connectivity. In the middle ear approach, the neurons are not cut. Supported by NIH NINCDS grant #DC00434.

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Acid-salt, Sucrose, and Quinine Sensitive Fibers in the Glossopharyngeal Nerve of the Rat. MARION E. FRANK (University of Connecticut Health Center, Farmington, CT)

The glossopharyngeal nerve innervates many hundreds of taste buds in two receptive fields: the foliate and circumvallate papillae in mammals. Nerve-cut experiments have shown the glossopharyngeal to be important in supporting immediate ingestive reflexes as well as long-term intake of both non-preferred and preferred taste solutions in rats. Yet few studies have assessed response properties of single glossopharyngeal nerve fibers. Of 75 recorded rat glossopharyngeal fibers that were neither mechanosensitive nor thermosensitive, 59 were taste-responsive (responding at rates at least 2.5 times as fast to a taste solution as to the water solvent for 5 s). Of those, 32 were sensitive to stimulation of the circumvallate and 27 were sensitive to stimulation of the foliate papillae. Solutions were introduced into a papilla via the tip (100-200 um) of a glass pipette, a method of stimulation that yielded responses of short latency and high rate. Mid-range concentrations of stimuli were chosen based on whole-nerve responses. Hierarchical cluster analysis of response (nerve impulses/ 5 s) patterns of fibers across stimuli defined three broad categories: 26 fibers showed A patterns, 19 Q patterns, and 14 S patterns. The spectrum of response patterns recorded from fibers with foliate receptive fields did not differ from the spectrum recorded from fibers with circumvallate receptive fields. Fibers with A patterns responded to HCl, acetic acid, citric acid, NH_4Cl, KCl, and NaCl. Fibers with Q patterns responded most consistently to quinine.HCl, $MgSO_4$, KCl, and Na saccharin. Fibers with S patterns responded to Na saccharin, sucrose, and fructose. Neither sucrose octaacetate, caffeine, nor urea consistently affected any of the taste-responsive fibers. The spectrum of response patterns for glossopharyngeal nerve fibers contrasts sharply with the spectrum reported for the chorda tympani nerve, which innervates taste buds in fungiform papillae on the anterior tongue. Thus, glossopharyngeal and chorda tympani nerve fibers provide the rat with qualitatively different information regarding chemicals in different parts of the oral cavity.

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Depolarizing Responses of Taste Cells To Chemical and Electrical Stimuli at the Apical Pore in Slices of Necturus Lingual Epithelium DOUGLAS A. EWALD and STEPHEN D. ROPER Dept. of Anatomy & Neurobiology, Colorado State U., Ft. Collins CO 80523 and the Rocky Mountain Taste and Smell Center, Denver CO 80262

Taste buds in Necturus contain three morphologically distinct cell types: dark and light receptor cells with apical ends extending to the surface of the lingual epithelium and basal cells which are located at the base of the taste bud. Receptor cells exhibit generator potentials and action potentials in response to tastants (Kinnamon & Roper, J. Physiol. 383 601, 1987). Chemical synapses have been identified ultrastructurally in the basal region of the taste bud between taste cells and sensory axons and also between adjacent taste cells (Delay & Roper, J. Comp. Neurol. 277 268, 1988). We have used transverse slices of epithelium (200 um in thickness) that contain taste buds to test for synaptic interactions among cells in the bud. The apical extremities of all the receptor cells in each taste bud were depolarized by focal application of brief pulses of high K+ solution (140 mM) delivered from a micropipette (20 um tip diameter). Recordings in the basal region of the taste bud had various latencies compared to the receptor potential recorded at the apical pore. Cells identified as receptor cells by Lucifer Yellow fills had responses with latencies less than 75 msec whereas cells identified as basal cells had latencies greater than 75 msec. The entire receptor cell population was also depolarized electrically by applying a large diameter (20-40 um) fire-polished glass stimulating electrode to the pore with gentle suction. As with chemical stimulation, electrical stimulation produced depolarizations in all the cells in the basal region. One component of this depolarization had a slow time course (secs), was labile to repetitive stimulation, and recovered with a time course of minutes. Its slow time course was consistent with the long latency of responses of basal cells to chemical stimulation and both phenomena may result from synaptic transmission from receptor cells to basal cells.
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Amiloride-Sensitivity of the Chorda Tympani Response to NaCl in Fischer-344 and Wistar Rats. ILENE L. BERNSTEIN (University of Washington), ALISON LONGLEY (University of Washington)

Fischer-344 (F-344) rats fail to prefer NaCl solutions to water at any concentration, and avoid NaCl solutions preferred by other strains, including Wistars. The NaCl aversion of F-344 rats is evident not only in 24-hr, two bottle tests but also using brief exposure, *taste reactivity* assessment. Behavioral and electrophysiological responses of the mammalian gustatory system to NaCl have been shown to depend on a sodium transport system which is specifically blocked by lingual application of the sodium transport blocker amiloride. We hypothesized that strain differences in sodium-transport mechanisms sensitive to amiloride could be involved in the F-344 rat's dislike of NaCl solutions. The present study examined whether strain differences exist between F-344 and Wistar rats in the amiloride-sensitivity of the chorda tympani (CT) electrophysiological response to NaCl. Whole nerve CT recordings were obtained from adult F-344 (N=12) and Wistar (N=10) rats (Simonsen, Gilroy, CA) during chemical stimulation of the anterior tongue. A NaCl concentration series ranging from .01M to 1.0M was examined both before and after pretreatment with a 5 uM or 500 uM solutions of amiloride hydrochloride. Integrated whole nerve responses to NaCl solutions were expressed relative to the response to .5M NH_4Cl. Strain differences in the response to NaCl solutions emerged, with F-344 animals showing a significantly larger response to NaCl relative to NH_4Cl than Wistars. This strain difference was completely eliminated by amiloride pretreatment, which suppressed the electrophysiological response to NaCl in both strains, but had a proportionally greater effect in the F-344 strain. The implication of these findings with regard to the aberrant NaCl preference seen in F-344 rats will be discussed.

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Mixture Suppression Toward Binary Odorant Mixtures in Spiny Lobsters: Behavioral Assay using the Antennular Flick Response. PETER C. DANIEL AND CHARLES D. DERBY (Dept. of Biology, Georgia State University, Atlanta, GA)

Lobsters respond behaviorally to chemical mixtures whose constituents excite olfactory receptor cells in the antennules. The behavioral response to a mixture should be predictable from knowing 1) the behavioral responses to components of the mixture and 2) characteristics of the receptor cell population. We are investigating the accuracy of our predictions of behavioral responses to binary mixtures based on the responses to individual chemicals (AMP, betaine, cysteine, succinate, and taurine) and the responses to binary combinations of these chemicals using an antennular flick assay. Three models (substitution, polynomial, and mixed) were used to compute predicted responses to mixtures. Observed deviations from these predictions are defined as mixture interactions. The first two models assume that receptor cells with identical response properties have either one receptor type which responds to both chemicals (substitution) or two receptor types each of which responds exclusively to only one of the chemicals (polynomial). The mixed model combines elements of both of the above models according to our knowledge of receptor cell types from electrophysiological studies of response specificity and cross-adaptation for 229 antennular olfactory cells. For each binary mixture, three receptor cells can be identified and incorporated into the mixed model: 1) specific receptors to only one chemical, 2) two receptors specific for each of the two chemicals, and 3) one receptor for both of the chemicals in the binary mixture. The population response was determined from the average of the predicted responses of the 229 cells. If the predictions of the substitution model and the polynomial model are considered as lower and upper bounds respectively, then no interactions are apparent since observed reponses to the mixtures fall within these bounds. However, using the mixed model, observed reponses were suppressed for eight of the 10 binary mixtures: AMP:Bet, AMP:Cys, AMP:Suc, AMP:Tau, Bet:Cys, Bet:Suc, Cys:Tau, and Suc:Tau.

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Fine Structure of Aquatic Odor Plumes in Laboratory and Deep Sea Conditions. JELLE ATEMA, PAUL A. MOORE, LAWRENCE MADIN*, and GREG A. GERHARDT (B.U.M.P., M.B.L., Woods Hole, MA 02543 USA, W.H.O.I., Woods Hole, MA 02543 *Department of Psychiatry and Pharmacology, University of Colorado, Denver, CO, 80262)

Odor plumes serve as sources of information for many animals during chemically mediated orientation. The information contained within the odor plume is poorly understood because the spatial and temporal scales at which chemoreceptor cells and organs function have been difficult to match with conventional chemical detectors. With newly introduced electrochemical microelectrodes (Moore et al. 1989, Chem. Senses 13: in press), we can sample certain chemical tracers, such as dopamine, at micrometer space scales and millisecond time resolution. We used this high resolution recording technique to sample the fine scale structure of an odor plume under a) laboratory and b) deep ocean conditions. The laboratory plume was created in a uni-directional seawater flume (90 x 250 x 20 cm). After allowing the plume to establish itself for 2 min, an odor profile was recorded by sampling odor concentrations at 10 Hz at 63 sites each for three minutes using a graphite-epoxy capillary electrode and a computer-based recording system (IVEC-5). The sample sites were located in a three-dimensional array downstream. The oceanic plume was created using a pressure release system located on a near-bottom platform at 1,000 m. This plume was sampled over a 50 m track at 18.75 Hz by Subnose-I, a portable, compact version of the IVEC-5 mounted on the Johnson Sealink submersible. Analysis shows that spatial gradients of certain odor pulse parameters (Moore and Atema, 1988; Biol. Bull. 174:355-363) point to the source. Specifically, the frequency of large peak heights and large onset slopes increase toward the source, whereas other parameters decrease and yet others remain constant. Animals may use this gradient information during orientation behavior and their chemoreceptor filter properties may be "tuned" to certain odor pulse parameters.

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Odor Perception in a Marine Predator. RICHARD K. ZIMMER-FAUST (Marine Environmental Sciences Consortium and Department of Biology, University of Alabama, Dauphin Island, AL 36528)

The California spiny lobster, *Panulirus interruptus*, is a useful animal for investigating the relationship between chemoreception and foraging, because it has olfactory and taste receptors that are highly sensitive to compounds released by prey, and because it responds actively to chemical stimuli under field and laboratory conditions. Mollusks are the preferred prey of lobsters, and in field experiments, lobsters are attracted significantly more often to the scents of gastropod and bivalve mollusks than to polychaete worms, sea urchins, crustaceans or fishes. Field tests show that the principal attractants in gastropod flesh are > 1000 daltons molecular mass, though compounds, < 1000 daltons, are also attractive. Lobsters are repelled by the scents of predators, competitors and freshly killed conspecifics, and lobsters only move away from protective burrows at night to feed when most of their predators are resting and inactive. The primary effect of prey odor is to modify the locomotory patterns of lobsters that are endogenously active; prey odors have potencies that are several orders of magnitude lower to lobsters resting either inside or outside of burrows. Finally, results indicate that chemoreception probably mediates foraging by lobsters at distances less than 0.5 - 1.0 meter away from prey patches on shallow, sub-tidal rocky reefs, therefore, endogenous patterns of locomotion are of primary importance to the successful predation by *P. interruptus*.

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Chemosenory Similarity of Amino Acids and Other Low Molecular Compounds by Catfish. VALENTINCIC T.*, OTA D., BLEJEC A., and METELKO J. (Department and Institute of Biology, University of Ljubljana, Yugoslavia).

Feeding responses in naive brown bullhead catfish *Ictalurus nebulosus* are only occasionally released by single chemicals. Thirty conditioning sessions with a food reward terminating each session were necessary to establish a regularly occuring food search to a previously neutral stimulus. After the establishment of the association between the amino acid and the food reward, the animal searched for food in response to any stimulus perceived as similar to the conditioned stimulus. Theoretically, a substance is perceived as similar if it releases the same patterns of electrical activity in the same receptor cells. Although there is no information on single receptor cell activity in the olfactory system of ictalurid catfish, single taste unit studies in *Ictalurus punctatus* indicated a specialization of taste receptor cells (Kohbara et al, AChemS XII, 1989). The present similarity experiments were designed to complement recent electrophysiological and biochemical studies concerning independence of receptor sites. L-methionine, L-alanine, esters of L-alanine, and L-norvaline released intense searching behavior in L-norleucine-conditioned bullheads, while neutral amino acids with branched side chains and L-arginine had no effect. Subsequent to L-alanine conditioning, L-alanine esters, glycine, β-alanine, L-serine, L-cysteine, and D-alanine released food search, whereas substances such as L-arginine, L-proline, and L-leucine were ineffective. Cross-reactivity between neutral amino acids with short side chains and those with long unbranched side chains was observed in both cases. Conditioning to L-cysteine induced responsiveness to its esters and DL-homocysteine, while other amino acids were ineffective. L-lysine was the only partially stimulatory substance after L-arginine conditioning, suggesting the existence of a highly specific arginine receptor in a second ictalurid species.

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Carnosine Synthetase Immunoreactivity in the Olfactory Epithelia of Amphibia. Maria J. Crowe & Sarah K. Pixley, Dept. of Anatomy & Cell Biology, Univ. of Cincinnati 45267

The dipeptide carnosine has been found in muscle and/or nervous tissue of various mammalian, avian and amphibian species and is particularly enriched in olfactory tissues. The function of carnosine in olfactory tissues is unknown, although it may serve as a neurotransmitter or neuromodulator in primary olfactory neurons (PONs). To further examine this putative role of carnosine in the olfactory pathway, previous investigators have focused on the properties and localization of its biosynthetic enzyme, carnosine synthetase (CS). Monoclonal antibodies (Mabs), which were generated against rabbit muscle CS, were found to form immune complexes with olfactory epithelia (OE) and olfactory bulb (OB) tissue extracts from various species (Margolis et al, J. Neurochem. 48:593-600, 1987). The use of these Mabs for immunocytochemical localization in tissues or on immunoblots from the same species has not been successful to date. We have used the Mab CS E (gift of FL Margolis) for immunocytochemical studies of Rana pipiens, Rana catesbiana and Ambystoma tigrinum. We report here that we have observed specific immunostaining of subsets of OE cells in tissue sections from R. pipiens and R. catesbiana, but not from A. tigrinum. Labelled cells in the Rana OE morphologically resembled PONs. Dendritic-like processes extended to the luminal surface and ended in brightly labelled knobs. Axonal processes extended to and through the OE basal lamina, ending in the lamina propria. Only a subset of PONs present in any one section were labelled. This is the first study demonstrating immunocytochemical localization of CS in amphibian OE cells. Other investigators have used Mabs to carnosine to localize the dipeptide itself in amphibia retina cells (Fasolo et al, Neurosci. Abst. 15:154.6, 1989) and in rat OE cells (Sakai et al, Experientia 43:298-300, 1987). These findings, combined with our own, indicate that the amphibian OE can be a useful system for study of the possible neural functional role of carnosine and its biosynthetic enzyme.

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Transplantation of embryonic cat nasal tissue into adult cat spinal cord. S. K. PIXLEY (Univ. of Cincinnati, Cincinnati, OH), D. ANDERSON (Veteran's Administration, Cincinnati, OH) and P. REIER (Univ. of Florida, Gainesville, FL).

Embryonic cat nasal tissues, from 4 embryos of day E24, were dissected free of external tissue. The nasal pit was a simple round sac surrounded by mesenchyme, without cartilage or bone. These tissues were placed in a lesion made in one side of the upper lumbar spinal cord of an adult cat. The lesion was an expansion of a previous crush injury site, an injury which mimics chronic spinal cord damage. The adult cat was monitored closely for return of function and signs of discomfort. No discomfort was ever seen, but function became impaired 6 weeks after the operation. The transplant material was removed, examined superficially, sectioned and then immunostained with several cell-type-specific antibodies. Several extensive cavities were found in the transplant area in the cord. The inner linings of these cavities were infolded, as in nasal tissues of older animals than E24. Cartilage had formed in shapes resembling turbinates, and areas of bone formation were seen. Three types of epithelia lined cavities: olfactory-like, respiratory-like and keratinizing. Structures resembling hairs were seen. Immunostaining showed rare OMP-positive cells. Immunostaining with SUS-1, specific for sustentacular cells, showed staining only in areas corresponding to those stained with OMP. Silver staining showed that several epithelial areas that were OMP-negative, still contained cells resembling olfactory receptor neurons. Immunostaining with anti-GFAP, anti-neurofilament protein and anti-myelin basic protein showed no glial scarring and suggested that host neuronal fibers were penetrating the transplant tissue. These studies are very promising, because the tissue was not rejected and glial scarring was not seen. It is therefore theoretically possible that beneficial host/graft interactions could occur.

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Survival of Olfactory Receptor Neurons in Dissociated Cell Culture is Enhanced by 2-Mercaptoethanol. R. J. GRILL and S. K. PIXLEY (Dept. of Anatomy & Cell Biology, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267).

The development of dissociated cell cultures containing olfactory receptor neurons (ORNs) has been difficult, in part, because of problems in identifying cell types, but also because the neurons have been difficult to support in dissociated cell culture. In a recently described system for culturing newborn Spraque-Dawley rat olfactory epithelial cells (Pixley and Pun, Dev. Brain Res., in press), neuron identification problems were solved by using four neuron-specific antibodies and patch-clamp techniques to identify neurons. In the process of developing these cultures, conditions were found which greatly increased survival of ORNs, including a specific culture medium which was originally developed for support of another cell type. The components of this medium have been analyzed in olfactory cell cultures. The key ingredient for increased neuronal survival was found to be 2-mercaptoethanol. The function of 2-mercaptoethanol in neuronal survival may be similar to its function as a growth factor for lymphocyte and hybridoma cells, which is to enhance cysteine uptake and decrease cysteine degradation in the medium. To study this mechanism, a serum-free medium and the appropriate substrate conditions have been established for ORNs. Other factors which have been found to be important in neuronal survival were conditions of dissociation, in particular the enzymes used and the degree of mechanical dissociation. Our cultured ORNs, which are in monolayers without a feeder layer of olfactory bulb astrocytes, are electrically active, respond to odors, but do not have OMP. This system should prove useful in determining ORN electrical properties and the function of OMP.

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Glutathione in the Olfactory Epithelium. C.L. KIRSTEIN, R.J. BRIDGES, R. COOPERSMITH and M. LEON. (Dept. of Psychobiology, University of California, Irvine CA 92717).

The olfactory receptor neurons of the nasal epithelium represent a potential site of entry for a wide variety of substances into the brain. Under most conditions, the brain appears to be protected from volatile chemicals that could enter in this manner, suggesting that efficient detoxification mechanisms exist in the olfactory system. To examine this hypothesis we measured glutathione (GSH) levels in various brain regions. GSH is an essential intermediate in several detoxification processes and therefore serves as a good marker for the presence of protective systems. We find enriched levels of GSH in the olfactory epithelium that are localized to the olfactory receptor neurons. Lower GSH levels were found in the olfactory bulb, hippocampus, cerebellum and cortex, respectively. Although GSH levels decreased in all tissues throughout the lifespan, the levels in the olfactory epithelium remained enriched relative to other brain regions. To examine the potential effects of the decreased levels of GSH during aging, we measured malondialdehyde, an end product of lipid peroxidation, as a general index of peroxidative damage. While malondialdehyde levels were consistently increased with age in all brain regions, they were significantly lower in the olfactory epithelium throughout the lifespan. The increased presence of GSH, in combination with other detoxification mechanisms (e.g., catalase, cytochrome P450) suggests that the olfactory epithelium is a specialized tissue which effectively deals with incoming xenobiotics. These systems may also be involved in the termination of chemical stimulation in the receptor neurons.

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Localization of the Cyanide Metabolizing Enzyme, Rhodanese, within the Olfactory Epithelium. JL LEVIS, CE RHOADES, DE BICE, JR HARKEMA, JA HOTCHKISS, and AR DAHL. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Hydrogen cyanide is a potent toxicant with a distinctive odor of burnt almonds. Inhalation exposure to hydrogen cyanide ranges from exposure to the lov concentrations released during cooking of cyanogenic fruits to the high concentrations released during combustion of nitrogen-containing synthetic materials. Olfactory detection of cyanide is an important warning system in environmental exposure to toxic levels, but the ability to detect cyanide is quite variable in the population. Inability to smell cyanide has resulted in several occupational deaths among emergency and medical personnel and has led Orange County, California to pair pathologists unable to smell cyanide with a partner who can. The activity of the cyanide metabolizing enzyme, rhodanese, is high in nasal tissue in rats and humans and may play a role both in reducing the concentration of cyanide at the olfactory receptors and in the detoxication of innaled cyanide. Biochemical analyses of homogenates from rat and bovine nasal tissue have shown rhodanese activity to be greatest in the ethmoturbinate or olfactory epithelium. The present study was designed to determine the cellular distribution of rhodanese within nasal tissues using immunohistochemical methods. Antibodies to bovine rhodanese raised in rabbits and shown to react with rhodanese in western blot analysis were incubated with paraformaldehyde-fixed sections from nasal pharynx, septum, proximal and distal maxilloturbinates, and ethmoturbinate. Ethmoturbinate tissue showed the greatest amount of immunostaining, a finding consistent with the rhodanese activity assays. The reaction was localized to cells of Bowman's glands. These findings are consistent with the localization of other xenobiotic metabolizing enzymes in nasal tissue and support our previous proposals that nasal enzymes function in both olfaction and xenobiotic detoxication.

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Effect of Olfactory Epithelial Regeneration and Cage Environment on the Sensitivity of the Rat Olfactory Epithelium to Methyl Bromide. BRAD BOLON, MARC BONNEFOI, KEVIN T. MORGAN (Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709).

Inhalation exposure of rats to methyl bromide (MeBr) induces selective olfactory epithelial degeneration with evidence of early regeneration during 5 daily exposures (Toxicol. Appl. Pharmacol. 94:311, 1988). The present study was designed to assess the sensitivity of more fully regenerated olfactory epithelium to a second MeBr exposure. During this study, the use of filter-cap cages combined with wood chip bedding was also found to induce olfactory epithelial lesions which had a clear influence on responses to methyl bromide. Four groups (n=3/group) of 16-week-old male F-344 rats were exposed for 6h to 0 (groups I, II) or 175 (III, IV) ppm MeBr, kept in filter-cap cages for 4 wks with weekly changes of bedding, re-exposed for 6h to 0 (I, III) or 175 (II, IV) ppm MeBr, and sacrificed 24h later. Additional control animals (n=2/group) were exposed once for 6h to 0 (V) or 175 (VI) ppm MeBr on day 1, kept in filter-cap cages for 24h, and sacrificed. Histopathologic examination of the nasal passages from all rats revealed very consistent housing- and treatment-related effects within each group. Housing in filter-cap cages for 4 wks (I to IV) was associated with chronic/active inflammation of the nasal vestibule and extensive olfactory sensory cell loss in the dorsal meatus; basal and sustentacular cells were generally intact. These lesions, which were absent in control rats housed for only 24h in filter-cap cages (V, VI), were considered to be the result of airborne contaminants (ammonia) from soiled bedding. This proposal was supported by concurrent studies in this institute which revealed ammonia concentrations in excess of 100 ppm under similar caging conditions, by the similarity of the nasal vestibular lesions to previously reported ammonia-induced lesions in rodents (Toxicol. Appl. Pharmacol. 74:417, 1984), and by the absence of such lesions in rats kept for only 24h during this study. Exposure of rats to MeBr revealed (a) extensive destruction of olfactory epithelium (as reported previously) in groups II to IV, including the epithelium of the dorsal meatus in rats without caging-associated olfactory lesions (VI); (b) resistance of the olfactory epithelium in the dorsal meatus (which exhibited caging-associated loss of sensory cells) to MeBr (II to IV); (c) extensive regeneration of olfactory epithelium 4 wks following a single MeBr exposure (III); and (d) partial to complete resistance of regenerating (previously MeBr-damaged) olfactory epithelium to a subsequent MeBr exposure (IV). This study clearly demonstrates the potential of prior toxicant exposure and/or caging conditions to dramatically influence olfactory epithelial responses to inhaled xenobiotics in a site-specific manner.

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Effects of unilateral naris closure on the olfactory epithelia of adult mice. JOEL MARUNIAK, JEFF HENEGAR, and T.P. SWEENEY (Biological Sciences, University of Missouri, Columbia, MO 65211

This study investigated the effects of unilateral naris closures of varying durations on the olfactory epithelia of adult mice. Naris closures were performed for 0.75, 2, 3, 4, 5, 6, 7 or 8 months. The effects were restricted to the open-side olfactory epithelium, were both time and position dependent, and were always most severe in the rostral regions. At all closure times the olfactory epithelia on the closed sides appeared to be normal when compared to control epithelia. The most commonly observed abnormalities were loss of receptor neurons and decreased epithelial thickness. The rostral third of the open-side olfactory epithelia showed neuronal losses at 2 and 3 months, regrowth at 4 months, and losses at 5, 6, 7 and 8 months of closure. The effects of naris closure on the morphology of the epithelium on the open sides were often striking. In mice that had significant losses of receptor neurons, the epithelium frequently had only supporting and basal cell layers with virtually the entire neuronal layer absent. Depending on the length of closure, the middle regions of the open side showed either no effect, losses, or else an increase in the number of receptor neurons. Naris closure resulted in an increase in the number of receptor neurons in the caudal regions of the open side at all time points. In the 7 and 8 month closure groups, 2 additional abnormalities were observed on the open sides. Some animals showed an increased rugosity of the olfactory epithelial surface. In others large numbers of polymorphonuclear leukocytes were visible on the epithelial surface indicating active inflammation.

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Two-Dimensional Models and Morphometry of Individual Olfactory Conchae in Growing rats. MICHAEL SICHLAU, MARK PATERNOSTRO AND ESSIE MEISAMI (Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801)

We recently reported (Meisami et al Neurosci. Abst. 15:291,'89) on development of different kinds of 2-D fold out models to represent the mechanism and general growth plan of olfactory epithelium (OE) in growing rats from ages 1 to 90 days. Using morphometric means, we concluded that the surface area (SA) of OE grows along its entire expanse. To obtain exact information on the variation and uniformity of growth rates in different OE regions, more detailed measurements were necessary. In this report, we apply the same approach to delineate and morphometrically measure growth of OE in each individual concha (I-VI) and nasal septum. Results indicate that at birth all conchae are present but the superior ones are more developed than the inferior ones; conchae V and VI are very rudimentary. At 90 days, the largest primary concha is # III from the top, followed by conchae I, II, VI, V and IV. OE growth occurs in the septum and in all conchae but at different rates. At birth septal OE is 33% of total, declining to 18% at 90 d. In concha III, the largest, 15% of total SA is present at 1 d, and 25% by 90 d. In 90 d rats, the smallest conchae is # IV from the top with 5% of total SA; this value is the same at birth. Concha # VI is barely visible at birth, growing to 3% of total SA at 10 d, and 12% by 90 d. Growth ratios, defined as (90d SA/1d SA), were found to be 13x for the septal OE and Concha I, 32x for conchae II and III, 60x and 120x for conchae V and VI respectively.

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Voltage-Dependent and Stimulus-Activated Membrane Currents in Isolated Olfactory Neurons of the Channel Catfish (Ictalurus punctatus). TAKENORI MIYAMOTO, DIEGO RESTREPO and JOHN H. TEETER (Monell Chemical Senses Center, Philadelphia, PA. 19104, USA)

An odorant regulated cyclic AMP cascade has been implicated in olfactory transduction. Recent studies in the catfish, using amino acids as stimuli for specific receptor proteins, have also implicated the involvement of phosphoinositide turnover in olfactory signal transduction (see accompanying abstract-Teeter et al). We have studied the electrical properties of isolated catfish olfactory neurons with whole-cell patch clamp to examine the mechanisms underlying olfactory transduction. Olfactory neurons were isolated from the catfish olfactory epithelium by dissociation with papain in divalent cation-free Ringer. In normal fish Ringer, the resting potential of isolated olfactory neurons varied from -25 to -76 mV and the input resistance ranged from 1 to 5 GΩ . Membrane capacitance was approximately 2 pF in these small cells. All olfactory neurons examined displayed a voltage-dependent inward Na^+ current which was blocked by 1 uM TTX or by substitution of $NMDG^+$ for Na^+, and an outward K^+ current which was suppressed by external Ba^{2+} or internal Cs^+ substituted for K^+. The amplitude and time course of the outward current varied from cell to cell. In most cells, a small sustained inward current was measured with 10 mM Ba^{2+} in the bath. This current resembled the L-type Ca^{2+} current observed in other neurons. Some, but not all isolated olfactory neurons responded to a mixture of amino acids with an inward current at normal resting potential. These currents are consistent with either cyclic AMP- or IP_3-mediated increases in membrane conductance.

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Mechanism of Olfactory Signal Transduction in Primary Cultures of Rat Olfactory Neurons. Gabriele V. Ronnett[1,2], Lynda D. Hester[1], Solomon H. Snyder[1] ([1]Department of Neuroscience and [2]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.)

An understanding of the mechanism(s) olfactory signal transduction has been hindered by inability to obtain homogeneous cultures of neurons for biochemical investigation. We have developed a method for culture of neonatal rat olfactory neurons. These cells are bipolar in culture, and stain positively for neuron specific enolase, olfactory marker protein (OMP), vimentin, but are negative for glial fibrillary acidic protein (GFAP), S-100. These cultures are greater than 98% homogeneous. These cultures demonstrate robust adenylate cyclase activity and phosphodiesterase activities. Exposure to cells to odorants causes a rapid, transient, dose-dependent rise in intracellular cyclic AMP levels. This increase is seen in pleasant and putrid odorants. To confirm that indeed adenylate cyclase activity is increased, thereby resulting in increased cyclase AMP levels, in situ adenylate cyclase acids were performed in culture wells. These experiments demonstrated that odorants caused increases in adenylate cyclase activity. Other studies have demonstrated changes in phosphoinositide turnover upon odorant exposure. Therefore, olfactory signal transductions appears to be complicated and potentially involve multiple second messenger systems.

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Activation of Inositol-Phosphate Metabolism in Primary Olfactory Cell Cultures. Susan F. Wood, Gabriele V. Ronnett, and Solomon H. Snyder (Dept. of Neuroscience, Johns Hopkins Univ.Sch. of Med., Baltimore,MD 21205)

We have recently reported the development of a primary olfactory neural cell culture from neonatal rat which rapidly responds to odorants by producing cAMP (Ronnett et al.,Neurosci. Abs.,p.749,1989). However the possibility exists for other second messenger pathways to be stimulated either directly or secondarily by odorants. To test this hypothesis, we have begun additional studies on olfactory transduction by measuring changes in inositol mono-phosphates ($InsP_1$) and inositol polyphosphates ($InsP_2$, $InsP_3$, and $InsP_4$) over time and in response to odorant stimulation. Cells were labelled with ^3H-inositol for 24 hours before stimulation with a variety of odorants in the presence or absence of LiCl. The stimulation was quenched using TCA and inositol phosphates analysed using Dowex chomatography or HPLC. We have observed changes in inositol phosphate levels as early as 1 s after stimulation with concentrations as low as 1 uM odorant. This suggests that the inositol phosphate pathway may be involved in olfactory transduction.

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Dynamics of Cyclic AMP Regulation in Olfactory Cell Lines. FELICE F. BORISY[1], RENE HEN[2], GABRIELE V. RONNETT[1], SOLOMON H. SNYDER[1] ([1]Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205. [2]Howard Hughes Medical Institute. Columbia University, New York, New York 10032)

Olfactory receptor neurons may be individually responsive to particular classes of odorants. Immortalized cell lines present an opportunity to generate large homogeneous populations of cells derived from an individual clone. A collection of cell lines, each of which is best responsive to a different set of odorants, may be used to elucidate the mechanisms of olfaction. We have studied the effect of odorant stimulation on cAMP regulation in immortalized neuronal cell lines derived from olfactory epithelium. Cells grown in DMEM + 10% FBS were used immediately prior to confluence. Measurement of adenylate cyclase activity in the 13S.1.22 cell line indicated a basal activity of 13.5 pmol/mg/min and a GTPγS stimulatable activity of 70 pmol/mg/min. Cells were stimulated by a variety of odorants including isovaleric acid, galbazine, citralva , s-carvone and thiazole. Changes in cellular levels of cAMP were measured by radioimmunoassay. Basal level of endogenous cAMP was 60.5 pmol/mg. Dose-dependent alterations in total cAMP were observed. Odorant stimulation produced up to a seventy percent increase in cAMP. This response to odorants was also studied in cells previously treated by differentiation causing agents, specifically retinoic acid or isobutylmethylxanthine. In addition, the cells were grown on several substrates to assess the role of extracellular matrix in an olfactory receptor cell's ability to respond to odorants.

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ODORANT BINDING PROTEIN: POSSIBLE BINDING SITE AND MULTIPLE SITES OF PRODUCTION. A.A. Khan and S.H. Snyder. (Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med. Balt., MD 21205)

The lateral nasal gland (LNG) is the major source of odorant binding protein (OBP) which may be a carrier for odorants. The protein has been shown to bind odorants of various structural classes such as pyrazines, terpenoids, and aromatics. OBP is significantly homologous to a superfamily of proteins that are believed to transport hydrophobic ligands. This homology has suggested the possibility of a receptor binding site for the complex of odorants and OBP. Preliminary autoradiographic studies, with metabolically labelled [^{35}S]-met OBP, show a specific localization of OBP binding to olfactory epithelium and not to adjacent brain regions. These preliminary results indicate the existence of a binding site in olfactory epithelium for OBP. Sagittal sections of the removed gland and olfactory epithelium show novel aspects of glandular anatomy: multiple ducts projecting from the gland anteriorly and posteriorly; a projection from the gland to the tear ducts; and regions of glandular tissue confluency with olfactory epithelium. Preliminary immunocytochemical findings suggest the presence of OBP in sub-epithelial glands of the olfactory epithelium. Studies are underway to elucidate a possible binding site for OBP as well as to determine its alternative sites of production.

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Responses in Slices of Olfactory Epithelium Visualized with Voltage Sensitive Dyes and Video Image Analysis. J.N. BROUWER, P. FARMER, C.S. LUO & R.C. GESTELAND (Department of Anatomy & Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267).

Optical recording of membrane potential-dependent fluorescence changes provides a means of detecting the electrical activity of cells. The technique is especially suited for the study of small cells and for simultaneous observation of the activities of many cells in tissues. We report here the first optical measurements on olfactory receptors at magnifications high enough to resolve cellular elements.

Cells in 100 μm thick slices of olfactory epithelia of the frog *Rana pipiens* and the tiger salamander *Ambystoma tigrinum* were loaded with the styryl dye RH 414 (Molecular Probes, Inc) and observed with an inverted microscope with a 40x oil immersion objective. Emitted fluorescence was captured by a CCD video camera. Black offset and video gain were adjusted for maximal sensitivity to brightness differences within the frame. Image analysis hardware and software was used to perform algebraic operations on the acquired images and to create pseudocolor displays to represent intensity differences. Isoamyl acetate, anisole and 1-butanol were presented as vapor puffs over the slice to eliminate tissue movement.

Depolarization induced by 0.6-1.2mM increases in $[K^+]_o$ decreased fluorescence intensity by 0.1-2% in various regions of the slice. Hyperpolarization induced by decreasing $[K^+]_o$ by 0.8mM increased fluorescence intensity by similar amounts. Odorant effects were displayed by subtracting the image taken just before from that acquired 1s after odor application. In the frog odors caused small changes, mostly depolarizations, in the soma and olfactory knob layers of the tissue. Cells in some areas responded. Cells in other areas did not. In the salamander odors evoked larger fluorescence changes. These occurred both near to the epithelial surface and in elongated structural elements reaching from the surface to the basement membrane. Often there were large responses in cell apical processes. Different odors affected different cell populations. Responding elements were widely distributed across the slice.

We believe that this technique will be useful for studies of odor response profiles of receptor neurons.

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Rapid Kinetics of Second-Messenger Signalling in Olfaction. H. BREER, I. BOEKHOFF, E. TAREILUS (Institute of Zoophysiology, University Stuttgart-Hohenheim, 7000 Stuttgart 70, FRG.

Recently, biochemical and neurophysiological evidence has accumulated implicating that second messengers may provide the critical link between the initial odorant-receptor activation and subsequent ion channel activity of olfactory neurons. However, a second messenger must satisfy several criteria to be the internal transmitter for the initial stage of olfactory signal transduction. One major constraint is the kinetic of second messenger formation and decay; the synthesis of an internal transmitter must precede the time course of changes in membrane permeability and the second messenger signal must decay very rapidly. Using a rapid kinetic methodology and radiochemical binding assays, the odorant-induced formation of second messengers (cAMP, IP_3) in insect antennae and rat olfactory cilia was monitored in the subsecond time range. The application of odorants induced a very rapid and significant increase in either cAMP or IP_3 concentrations, reaching a peak within about 50 msec; thereafter, the elevated level rapidly declined to the basal concentration usually within 200 msec. Thus, the rapid and transient changes in second messenger concentration represent a molecular signal with a kinetic sufficiently rapid to mediate the odorant-regulated permeability mechanism in olfactory receptor cells. Whereas in insect antennae, pheromones induced exclusively the formation of IP_3, in rat olfactory cilia a selective accumulation of either cAMP or IP_3 was observed after stimulation with individual odorants. However, the two different second messenger pathways could not be correlated to classes with different odor quality. In all cases, the odorant-induced rapid formation of second messengers was found to be mediated by G-proteins, suggesting the existence of specific odorant-activated receptors. The rapid kinetic as well as the transient nature of the second messenger signals favour the concept that receptor-activated chemosensory reaction cascades are involved in the chemo-electrical transduction of olfactory receptor cells.

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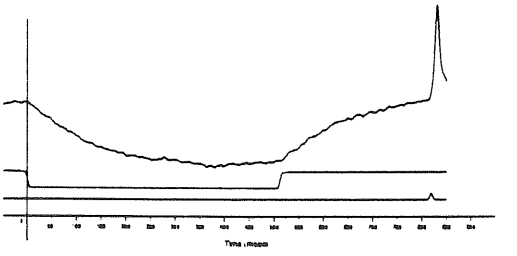
Biophysical Properties of Amphibian Olfactory Receptor Axons: *Semi-Quantitative Analysis Using Optical Recording Techniques*. RAYMOND GALINDO, PATRICK NASH, DAVID SENSEMAN, SYLVIA VASQUEZ (Brain Research Lab, The University of Texas at San Antonio)

Beginning with Rall & Shepherd's work in the late 1960's, a number of attempts have been made to develop mathematical models of neural processing within the vertebrate olfactory bulb. Such models have been based primarily on evoked extracellular field potentials supplemented with data provided by intracellular recordings from larger neuronal somata (e.g. mitral and tufted cells). Critical evaluation of extant models as well as the construction of new models might be facilitated if more was known about the intracellular potential changes that occur within the dendritic and axonal processes that are too fine to permit impalement with conventional intracellular microelectrodes. To this end we are using optical recording techniques to characterize the biophysical properties of neuronal cell types in the mudpuppy (*Necturus*) olfactory bulb beginning with the incoming axonal fibers of the primary olfactory receptors. The trace shown below was recorded optically in a single trial from a mudpuppy olfactory nerve following a 5.0 volt, 500 msec hyperpolarizing current pulse followed 625 msec later by a 100 volt, 0.8 msec shock to the nerve. From this and similar records we have calculated the following parameters for olfactory nerve axons: (a) conduction velocity = 0.13 m/sec, (b) space constant = 6.7 mm, (c) time constant = 0.14 s.



In the presence of 50 mM Na^+ propionate (which electrically uncouples glial), we observed measureable increases in both the space constant (11.1 mm) and the time constant (0.18 s).

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Examination of the Lineage of Adult Olfactory Receptors by Autoradiography and Intracellular Injection of Vital Dyes. M.A. SCHWARTZ and J.S. KAUER (Section of Neuroscience, Dept.of Anatomy and Cell Biology, Tufts Med. Sch. and Dept. of Neurosurgery, N.E.M.C., Boston, MA).

The present studies were designed to examine the lineage of developing olfactory receptor cells. Initial studies combined immunocytochemistry and 3H-autoradiography to characterize precursor populations in the mouse olfactory epithelium. Cytokeratin antibody appears to label the horizontal basal cells and not the globose basal cells, yet both types are capable of proliferation. Using a pulse-fix labelling paradigm to determine the number of 3H-labelled, cytokeratin-positive and 3H-labelled, cytokeratin-negative cells at various intervals after olfactory bulbectomy, it was demonstrated that the globose basal cell population, but not the horizontal basal group, increases its proliferative rate after degeneration of the receptor cells suggesting they are in the lineage pathway of new olfactory receptor cells.

In a second set of experiments this turnover process was examined in a salamander preparation in order to follow the lineage of single globose basal cells to receptor cells over time in living animals using intracellular vital dye injection. For example, it is not known whether globose basal cells give rise only to receptor cells or also to sustentacular cells. In these experiments, the olfactory epithelium was exposed in anaesthetized salamanders and individual cells were impaled and filled by iontophoresis with either Lucifer yellow or the rhodamine-labelled dextran, D-1868. We have successfully labelled olfactory receptor and sustentacular cells with both vital dyes and have followed these cells for up to 5 days. Visualization of somata, axons, dendrites and cilia was possible throughout this period. With this technique, one can examine the progeny of the basal cells in the adult olfactory system, by following injected globose basal cells for extended periods of time.

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Voltage-sensitive Dye Recordings from the Salamander Olfactory Bulb after Global and Local Odor Stimulation. A.R. CINELLI and J.S. KAUER (Section of Neuroscience, Dept.of Anatomy and Cell Biology, Tufts Med. Sch. and Dept. of Neurosurgery, N.E.M.C., Boston, MA).

Activity patterns within the laminae of the olfactory bulb were observed by imaging a voltage-sensitive dye after overall or punctate odor stimulation of the epithelium.

With overall ventral epithelial stimulation, different odors evoked different spatio-temporal activity patterns. Signal time courses were characterized by a brief, small amplitude hyperpolarization, a period of depolarization and a subsequent longer duration hyperpolarization. The patterns varied with different odors but partially overlapped one another within any one lamina. Higher intensity stimulation increased the size of the activated areas and modified the response latencies and time courses, although relatively distinct spatial patterns were still seen for different odors. Changing the direction of odor presentation from rostral->caudal to caudal->rostral had little effect on these patterns. Conditioning odor applications with the same or a different odor altered the time course and spatial distribution of subsequent activity. With restricted epithelial stimulation, certain regions were seen which had lower thresholds for stimulating bulbar activity than had other regions. Application of the same odor to the entire epithelium elicited activity in similar bulbar regions. Bulbar activity at this same site could also be elicited by a second odor if it was delivered at high concentration to the epithelial region which had a low threshold to the first odor. These data indicate that there is a complicated spatial relationship between those local epithelial areas that have low thresholds for a particular odor and their bulbar projection targets, and that receptor selectivity partly depends on stimulus concentration. They further suggest that there are both divergent and convergent connections between the olfactory epithelium and bulb and that the odors tested here are represented by distributed patterns of bulbar activity evoked by differential activation of several subpopulations of receptors in the epithelium.

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Distribution of Globuli Cell Dendritic Arbors in The Crayfish Olfactory Midbrain. DE FOREST MELLON, JR. & VINESSA ALONES. Department of Biology. University of Virginia. Charlottesville

In crustaceans, olfactory afferents course proximally within the antennular nerve and synapse with interneurons in the olfactory lobe glomeruli. It is believed that one category of second order olfactory interneurons are the globuli cells - groups of small diameter neurons with uniformly spherical perikarya that are a consistent feature of the crustacean olfactory midbrain. We have used intracellular injection of Neurobiotin (Vector Laboratories, Inc.) to examine the distribution of globuli cell dendritic arbors within the olfactory and accessory lobes of the crayfish, Procambarus clarkii. Neurobiotin was injected iontophoretically with sinusoidally oscillating, positively-biased current into individual globuli cell bodies in desheathed, isolated crayfish brains. Brains with Neurobiotin-filled globuli cells were fixed in a glutaraldehyde-paraformaldehyde mixture, mounted in gelatin, and sectioned on a vibratome for processing with avidin-horseradish peroxidase complex. Stained, cleared, and mounted sections were examined microscopically. Each globuli cell typically branches to both the olfactory and accessory lobes and projects an axon within the ipsilateral olfactory globular tract. Each olfactory lobe branch bifurcates once, sending crescent-shaped dendritic arbors into opposite hemispheres. Accessory lobe dendritic arbors are much more extensive, branching many times to invade all parts of this structure, presumably supplying each spherical glomerulus. The different respective branching patterns are consistent with a possible analytical role for the globuli cells in the determination of excitation patterns among olfactory interneurons and, consequently, in the identification of complex odorant stimuli.

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Serotonin in the central olfactory system of the spiny lobster. M. SCHMIDT, E. ORONA, S. WAECHTER, B.-A. BATTELLE, B. W. ACHE. Whitney Laboratory and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

Serotonin-like immunoreactivity has been demonstrated previously in the olfactory brain of crayfish (Brain Res 403: 371). As a first step towards understanding the possible function of serotonin in the olfactory CNS of crustaceans, we biochemically verified the presence of endogenous serotonin and identified the serotonin-immunoreactive cells in the olfactory brain of the spiny lobster, a species for which the peripheral input is well-characterized. The olfactory lobes (OL) contain serotonin in a concentration of 5.4 ± 1.1 ng/mg wet weight. Polyclonal antibodies raised against serotonin-BSA-conjugate (Incstar) and visualized by the PAP method were used to localize serotonin in vibratome sections of the brain. Serotonin-immunoreactivity was found in about 20% of the small somata in the ventral paired mediolateral cluster (VPMLC). The neurites of these cells are extremely fine and branch inside the OL. None of the somata in the ventral paired posterolateral cluster (VPPLC) showed any specific immunoreactivity. Particularly interesting are 4 giant, paired neurons with somata in the ventral paired medial cluster (VPMC) that showed positive staining. The neurites of these cells transect the olfactory-globular tract (OGT) and arborize extensively in the OL (as well as the accessory lobe). One of the giant neurons has a particularly large neurite (25-50 μm diam.) that also arborizes in the OGT. In the OL, both the immunoreactive neurons of the VPMLC and the giant neurons project to multiple glomeruli. All glomeruli contain a dense mesh of darkly stained terminal arborizations, although it is not yet clear from which of the immunoreactive neurons they originate. The size of at least the giant neurons favors electrophysiological analysis of their individual projection pattern(s) and function(s) in the olfactory CNS.

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Pharmacological and Physiological Evidence for Histamine as a Neurotransmitter in the Olfactory CNS of the Spiny Lobster. E. ORONA and B. W. ACHE. (Whitney Laboratory & Depts. Zoology & Neuroscience, Univ. Florida, St. Augustine, FL 32086)

Previously we established that the olfactory lobes of the spiny lobster Panulirus argus are capable of synthesizing HA from its precursor and are innervated by HA-immunoreactive interneurons (AChemS XI). Here, we provide pharmacological and physiological evidence for HA as a putative neurotransmitter in the olfactory CNS. HA was perfused into the brain in an "isolated head" preparation, via the artery supplying the cor frontale. Different doses of HA and antagonists were delivered to the brain for 40 sec at 3 min intervals. Extra- and intracellular recordings were made from circumesophageal connectives and from olfactory neuropil. HA (1 mM-1 μM) primarily produced excitatory effects on spontaneous activity (59% of 34 cells), although inhibition (24%) and non-responses (18%) were also observed. The responses were graded, dose-dependent, and could be reversibly antagonized. Cimetidine, a vertebrate-derived H2 antagonist, was more effective than pyrilamine (H1 antagonist), although the dose required was up to two orders of magnitude higher than that of the agonist. In some cells, odor-evoked responses could be blocked by HA, indicating that the action of HA results in an inhibitory effect somewhere within the olfactory pathway. That a HA-gated chloride channel is present on olfactory receptor cells (McClintock & Ache, PNAS, 86: 8137, '89) allows that the inhibitory action of HA may be presynaptic to the primary afferent terminals. Pharmacological investigations of the role of HA in the lobster olfactory system should benefit from a detailed map of HA containing neurons and more information on the cholinergic (nicotinic) properties (cf. Hardie, J.Exp.Biol., 138: 221,'88; Bayer et al., J.Exp.Biol., 145: 133,'89) of invertebrate HA receptors.

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Differential Synaptic Processing on Apical Versus Lateral Mitral Tufted Cell Dendrites. M.T. SHIPLEY (University of Cincinnati) and D.S. ZAHM (St. Louis University).

A classical feature of olfactory bulb anatomy is the existence of synapses between the dendrites of mitral/tufted (M/T) cells and granule cells (GC's) in the external plexiform layer (epl). M/T cell dendrites can be pre- or postsynaptic to GC's and reciprocal synapses are common. M/T cells have two classes of dendrites: 4-6 lateral dendrites extend for considerable distances in the epl; a single apical dendrite extends, without branching, through the epl into a glomerulus where it ramifies extensively and is synaptically contacted by olfactory nerve axons and juxtaglomerular cells. M/T cells are excitatory to GC's and GC's are inhibitory to M/T cells.

Upon reflection, however, there is a potential flaw in this conceptualization. If the apical dendrite receives extensive synapses from GC's, then the safety factor for sensory signals conducted from glomeruli to the somata of M/T cells will be very low. This suggests that the classical description of synapses between GC's and M/T cells applies to the lateral but not the apical dendrites of M/T cells.

To investigate this hypothesis, rat olfactory bulbs were subjected to Golgi impregnation either en bloc or post-sectioning and the synaptic organization of identified apical vs. lateral M/T cell dendrites were compared by EM analysis. The average distances between incoming synapses on lateral dendrites was 8 um vs. 105 um for apical dendrites. This extrapolates to 1 synapse/69 um^2 surface area of lateral vs. 1 synapse/11000 um^2 surface area of apical dendrites. Thus there are, at least, 160 fold more synapses per unit area of apical vs. lateral dendrites.

These results indicate that the apical dendrite of M/T cells is virtually devoid of incoming synapses. Thus, signals from the glomerulus can be conducted to the M/T soma with a high margin of safety. The dendrites of M/T cells represent an extreme example of differential synaptic processing among CNS neurons.

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Somatostatin-like immunoreactivity in the rat accessory and main olfactory bulbs.
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By using the rapid Golgi procedure, we have previously described the morphology of the mitral/tufted cells and other neurons in the accessory olfactory bulb (AOB) of the rat (Takami & Graziadei, Brain Res. in press: AChemS XI, 1989). In order to understand the functional significance of these neurons, we have begun classifying them by immunohistochemistry. We report here the morphology and distribution of somatostatin (SOM) and somatostatin-28 (SOM-28) immunoreactive neurons in the AOB in comparison with those in the main olfactory bulb (MOB).

Rats were sacrificed with transcardial perfusion and the olfactory bulbs were cut on a vibratome or a cryostat. The sections were then incubated with SOM or SOM-28 antisera and processed by using the avidin-biotin complex method.

In the granule cell layer (GRL) of the AOB, both antisera heavily stained a small population of large multipolar neurons. However, only SOM-28 antiserum stained heavily a very few small neurons in the glomerular layer (GL) and lightly only some of the mitral/tufted cells.

In the MOB, both antisera heavily stained large multipolar neurons in the GL and the GRL, but not the mitral cells. On the other hand, a subpopulation of periglomerular-located cells with a small ovoid soma were heavily stained with only SOM-28 antiserum. Their profusely branched dendrite spread in the domain of one glomerulus. SOM-28 immunoreactive neurons in the GL of the AOB were similar in morphology to these neurons.

These data provide an example of chemical differences between the AOB and the MOB, and they show differential stainability of the neurons by SOM and SOM-28 antisera.
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Pharmacological Characterization of Dopamine Receptors in the Olfactory bulb. WILLIAM T. NICKELL, ANDREW B. NORMAN AND MICHAEL T. SHIPLEY (University of Cincinnati College of Medicine, Cincinnati, Ohio)

Neurons in the glomerular layer of the olfactory bulb contain the transmitters GABA and dopamine (DA). These cells are local neurons which form synapses in the glomerular layer and possibly in the subjacent external plexiform layer. In the olfactory bulb $GABA_B$ receptors are segregated into the glomerular layer and topical application of the $GABA_B$ agonist baclofen inhibits the response to olfactory nerve stimulation. The quantity and localization of DA receptor subtypes in the bulb has not been adequately described and available physiological studies do not suggest a clear function for this DA system. We have determined the quantity and localization within the bulb of D1 and D2 DA receptor subtypes.

Scatchard analysis of [^{3}H]SCH23390 and [^{3}H] spiperone binding to D1 and D2 dopamine receptors, respectively, demonstrated the absence of D1 dopamine receptors and a B_{max} of 15 pmoles/g tissue for D2 dopamine receptors in homogenates of olfactory bulb. All assays included ketanserin (40 nM) to preclude binding to 5-HT_2 receptors.

Since DA containing neurons in the bulb are confined to and ramify within the glomerular layer it was probable that the D2 receptors are also located in that layer; however, some external tufted cells contain DA and these may project to other regions of the bulb. Thus the localization of the D2 receptors was determined autoradiographically. These experiments demonstrated that D2 receptors in the bulb were primarily located in the glomerular layer.

In other parts of the brain, D2 receptors are inhibitory and function by a mechanism similar to that of $GABA_B$ receptors, possibly suggesting that GABA and DA in the glomerular layer might have similar functions. In isolated turtle olfactory bulb, however, DA agonists do not cause the depression of response to olfactory nerve stimulation produced by the $GABA_B$ agonist baclofen (Nowycky, et al, Neurosci 8, 717). Thus, $GABA_B$ and D2 receptors in the glomerular layer may be located on different cellular elements.

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Comparison of GABA-like Immunoreactivity in the Salamander Olfactory Bulb Using Two Antisera. K.A. HAMILTON (LSU Medical Center, Shreveport, LA).

In the salamander olfactory bulb, granule cells appear to provide a major source of mitral/tufted cell inhibition, but other sources of inhibition might also exist (K.A. Hamilton and J.S. Kauer, J. Neurophysiol., 59, 1736, 1988). Immunocytochemical staining has suggested that cells in the granule cell layer, external plexiform layer and periglomerular region might contain the inhibitory neurotransmitter GABA (K.A. Hamilton et al., Chem. Sens., 12, 663, 1987). In the present study, the distribution and specificity of GABA-like immunoreactivity in the salamander olfactory bulb was further examined using two different antisera (rabbit anti-GABA/BSA, Incstar; guinea pig anti-GABA/Limulus hemocyanin, Eugene Tech).

Similar staining patterns were observed with both antisera and resembled previous descriptions. In the PG region, dense, heavily-stained cell bodies and dendrites surrounded the glomeruli, which contained stained dendrites and punctae. In the EPL, dense, stained dendrites and punctae surrounded sparse, stained cell bodies. In the MCL, stained punctae surrounded large, unstained cell bodies and dendrites. In the GCL, stained punctae were visible between smaller cell bodies, many of which were stained.

With both antisera, staining of punctae was blocked by preabsorption with GABA conjugates. Light to moderate staining of cell bodies and dendrites usually remained in the periglomerular region, however, even with high conjugate concentrations, suggesting that this staining was to some extent nonspecific. Slightly more nonspecific staining was observed with the rabbit antiserum, and with fixatives containing >0.5% glutaraldehyde.

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GABAergic Modulation of EEG and Evoked Potentials In The Rat Olfactory Bulb. BARRY K. RHOADES and WALTER J. FREEMAN (University of California at Berkeley).

GABA is an intrinsic neurotransmitter of the external plexiform and glomerular layers of the vertebrate olfactory bulb. In the external plexiform layer GABA mediates granule cell inhibition of mitral cells at reciprocal dendrodendritic synapses. Negative feedback across these synapses produces the gamma range oscillations which dominate olfactory bulb field potentials. The glomerular layer contains GABAergic periglomerular interneurons and both $GABA_A$ and $GABA_B$ receptors. Electrophysiological evidence indicates that these interneurons have the dual roles of dynamic range compression of glomerular throughput and tonic modulation of mitral cell excitability. The GABA receptor specificities for these actions were investigated in the olfactory bulbs of barbiturate anesthetized rats with local microinjections of A, B, and nonselective GABA agonists and antagonists. EEG and averaged evoked potentials (AEPs) for lateral olfactory tract (LOT) and primary olfactory nerve (PON) stimulation were recorded from surface electrodes immediately over the microinjection site. AEP effects for baclofen and phaclofen confirmed glomerular layer $GABA_B$ involvement in both attenuation of PON glomerular throughput and tonic excitation of the deep mitral-granule cell oscillator. AEP effects for muscimol and bicuculine could be attributed mainly to $GABA_A$ action at both granule-mitral cell dendrodendritic synapses and deeper dendrosomatic synapses. Picrotoxin, (a GABA Cl^- channel blocker) attenuated both the glomerular layer excitation and mitral-granule cell oscillations. Ongoing bulbar EEG was not influenced by local microinjections of GABAactive substances, but could be profoundly altered by regional surface applications. This confirms earlier experimental and theoretical evidence that cortical EEG is globally organized and that this global organization can override local neurochemical changes.

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Modulation of Olfactory Seizures by Valproate and Pentylene Tetrazol. BARRY K.RHOADES, EDMUN W. LEO, and WALTER J. FREEMAN (University of California at Berkeley).

Valproic acid is an anticonvulsant which is clinically effective against petit mal seizures. Valproate potentiates GABA action by blocking its breakdown. Recent evidence suggests that specific binding of valproate is limited to the glomerular layer of the olfactory bulb. Pentylene tetrazol is a potent convulsant which can induce petit mal type seizures. This study investigates the effects of valproate and pentylene tetrazol on electrically induced seizures of the rat olfactory bulb and cortex which may serve as an animal model for human petit mal epilepsy. As is generally the case with petit mal seizures, each induced olfactory seizure is self-terminating within a short time (<60 seconds), is accompanied by a state of behavioral absence, and causes no pain to the animal or damage to brain tissue. Rats are chronically implanted with electrodes in the olfactory bulb, piriform cortex, and lateral olfactory tract (LOT). Olfactory seizures of the bulb and cortex are induced in unrestrained animals by high frequency stimulation of the LOT. Valproate or pentylene tetrazol will be administered systemically and the effects on seizure induction threshold, duration, and intensity will be recorded. The restriction of valproate binding to the glomerular layer suggests that it may be an effective pharmacological tool for uniquely potentiating periglomerular cell GABAergic actions and distinguishing them from the GABAergic actions of granule cells in the deeper bulbar layers. Consequently, valproate effects on olfactory EEG and evoked potentials will also be examined. Results of these studies will be presented and discussed in the context of current theories of epileptogenesis and olfactory system function.

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Intracellular Recordings of Rat Olfactory Bulb Interneurons. DAVID P. WELLIS and JOHN W. SCOTT (Department of Anatomy & Cell Biology, Emory University School of Medicine, Atlanta, GA 30322)

It is generally accepted that interneurons mediate synaptic processing in the glomerular and external plexiform layers in the olfactory bulb (OB). However, studies of odor responses by identified interneurons in the OB have not been reported. Studies of these cells are important for interpreting the response patterns of mitral and tufted cells. We have obtained intracellular recordings from 27 granule cells and 5 presumed periglomerular (PG) cells. Here we present their responses to both electrical and odor stimulation.

Granule cells showed spontaneous EPSPs and/or spikes, responded to electrical stimulation of both the olfactory nerve (ON) and lateral olfactory tract (LOT), and showed a variety of responses to odor stimulation. With paired LOT pulses, we observed suppression of spike(s) and EPSP response to the test stimulation, indicative of a reduction of mitral cell drive (disfacilitation). However, intracellular potential and conductance analysis also suggested the presence of an IPSP following LOT stimulation. About half of the twenty cells we recorded during odor stimulation showed excitatory spiking responses. These responses were either habituating (response to first sniff only) or nonhabituating (response to every every sniff). The depolarizations underlying both the spiking and non-spiking responses were transient with each sniff or sustained across all sniffs. One granule cell's activity was decreased with odor stimulation.

Our limited number of periglomerular cell recordings show that these cells were also spontaneously noisy. A long-duration hyperpolarization followed a spike and large depolarization or burst of spikes (20-30 msec in duration) produced by an ON shock. Odor stimulation produced ~500 msec duration bursts of action potentials in all 3 cells tested. Three partial fills supported the identity of these cells as PG cells.

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Olfactory Discrimination Learning is Unimpaired Following Depletion of Norepinephrine in the Cortex and Olfactory Bulbs by Injection of 6-Hydroxydopamine Into the Dorsal Noradrenergic Bundle. Loredana M. Harrison & Robert G. Mair. University of New Hampshire, Durham, NH. 03824

Olfactory discrimination deficits have been reported in conjunction with diminished indices of brain norepinephrine (NE) activity in humans with neurologic disease and animals with experimental lesions. We investigated the role of NE in olfactory learning and memory formation by training rats in an odor discrimination task and then lesioning the dorsal NE bundle with the toxin 6-OHDA. Animals (Exp.=18, Control=9) were trained on three different two-choice odor discrimination problems. The first two problems were trained before surgery. After recovery from the 6-OHDA treatment, performance was measured first on the second (previously acquired) discrimination and then on the acquisition and reversal of the third (novel) discrimination. Control and experimental animals were compared on each of these post-surgical tasks for: percent correct, total time sampling the S-, perseveration time, and total running time to complete 25 trial sessions. No significant differences were observed for any of these measures for any of the tasks. HPLC analyses of brain tissue showed significant depletion of NE in olfactory bulb and cerebral cortex. No such deficits were observed for serotonin or dopamine.
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Saliency of Olfactory Cues: The Blocking Phenomena. L. HASTINGS and J. EVANS, (Department of Environmental Health, Univ. of Cincinnati).

A number of studies have shown that for macrosmatic animals such as rats, olfactory stimuli function as highly salient cues. In particular, rats prefentially attend to odors when paired with lights or tones in various discrimination paradigms (Nigrosh *et al.*, *J. Comp. Physiol. Psychol.* 89:285-294, 1975). To investigate whether olfactory cues retain their saliency when classical conditioning procedures are employed, rats were trained on a conditoned suppression task and tested using Kamin's blocking paradigm. For one group, the original conditoned stimulus was a tone and the redundant stimulus was an odor. The stimuli were reversed for the second group. The rats were trained 5 days on the original stimulus alone, 5 days on the compound stimuli, and tested on the individual stimuli on day 11. There was no evidence that the olfactory cue was any more salient than the tone, i.e., both redundant cues showed evidence of being blocked. However, the design of the exposure chamber was such that the rats may not have experienced the odor as the redundant cue. The chamber design was altered and the study is being repeated. The results will be discussed in terms of central vs. peripheral blocking of the stimuli.

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